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(54) Title: METHYLATED CpG ISLAND AMPLIFICATION (MCA) (57) Abstract A method is provided for identifying a methylated CpG containing nucleic acid including contacting a nucleic acid with a methylation sensitive restriction endonuclease that cleaves unmethylated CpG sites, contacting the sample with an isoschizomer of the methylation sensitive restriction endonuclease, wherein the isoschizomer cleaves both methylated and unmethylated CpG sites. The method also includes adding an oligonucleotide under conditions and for a time to allow ligation of the oligonucleotide to nucleic acid cleaved by the restriction endonuclease, and amplifying the nucleic acid. A method is also provided for detecting an age associated disorder including contacting a nucleic acid with a methylation sensitive restriction endonuclease that cleaves unmethylated CpG sites, contacting the sample with an isoschizomer of the methylation sensitive restriction endonuclease, wherein the isoschizomer cleaves both methylated and unmethylated CpG sites. The method also includes adding an oligonucleotide under conditions and for a time to allow ligation of the oligonucleotide to nucleic acid cleaved by the restriction endonuclease, and amplifying the nucleic acid. The amplified digested nucleic acid is adhered to a membrane, and hybridized with a probe of interest. A kit useful for detection of a CpG containing nucleic acid is also provided.		

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METHYLATED CpG ISLAND AMPLIFICATION (MCA)

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

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FIELD OF THE INVENTION

The present invention relates generally to regulation of gene expression and more specifically to a method of determining the DNA methylation status of CpG sites in a given locus.

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BACKGROUND OF THE INVENTION

DNA methylases transfer methyl groups from the universal methyl donor S-adenosyl methionine to specific sites on the DNA. Several biological functions have been attributed to the methylated bases in DNA. The most established biological function for methylated DNA is the protection of DNA from digestion by cognate restriction enzymes. The restriction modification phenomenon has, so far, been observed only in bacteria. Mammalian cells, however, possess a different methylase that exclusively methylates cytosine residues that are 5' neighbors of guanine (CpG). This modification of cytosine residues has important regulatory effects on gene expression, especially when involving CpG rich areas, known as CpG islands, located in the promoter regions of many genes.

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Methylation has been shown by several lines of evidence to play a role in gene activity, cell differentiation, tumorigenesis, X-chromosome inactivation, genomic imprinting and other major biological processes (Razin, A., H., and Riggs, R.D. eds. in DNA Methylation Biochemistry and Biological Significance, Springer-Verlag, New York, 1984).

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Human cancer cells typically contain somatically altered nucleic acid, characterized by mutation, amplification, or deletion of critical genes. In addition, the nucleic acid from human cancer cells often displays somatic changes in DNA methylation (E.R. Fearon, *et al.*, *Cell*, 61:759, 1990; P.A. Jones, *et al.*, *Cancer Res.*, 46:461, 1986; R. Holliday, *Science*, 238:163, 1987; A. De Bustros, *et al.*, *Proc. Natl. Acad. Sci., USA*, 85:5693, 1988); P.A Jones, *et al.*, *Adv. Cancer Res.*, 54:1, 1990; S.B. Baylin, *et al.*, *Cancer*

Cells, 3:383, 1991; M. Makos, *et al.*, *Proc. Natl. Acad. Sci., USA*, 89:1929, 1992; N. Ohtani-Fujita, *et al.*, *Oncogene*, 8:1063, 1993). However, the precise role of abnormal DNA methylation in human tumorigenesis has not been established. Aberrant methylation of normally unmethylated CpG islands has been described as a frequent event in immortalized and transformed cells, and has been associated with transcriptional inactivation of defined tumor suppressor genes in human cancers. In the development of colorectal cancers (CRC), a series of tumor suppressor genes (TSG) such as *APC*, *p53*, *DCC* and *DPC4* are inactivated by mutations and chromosomal deletions (reviewed in Kinzler and Vogelstein 1996). Some of these alterations result from a chromosomal instability phenotype described in a subset of CRC (Lengauer *et al.*, 1997a). Recently, an additional pathway has been shown to be involved in a familial form of CRC, hereditary non-polyposis colorectal cancer. The cancers from these patients show a characteristic mutator phenotype which causes microsatellite instability (MI), and mutations at other gene loci such as *TGF- β -RII* (Markowitz *et al.*, 1995) and *BAX* (Rampino *et al.*, 1997). This phenotype usually results from mutations in the mismatch repair (MMR) genes *hMSH2* and *hMLH1* (reviewed by Peltomaki, and de la Chapelle, 1997). A subset of sporadic CRC also show MI, but mutations in MMR genes appear to be less frequent in these tumors (Liu *et al.*, 1995; Moslein *et al.*, 1996).

Another molecular defect described in CRC is CpG island (CGI) methylation. CGIs are short sequences rich in the CpG dinucleotide and can be found in the 5' region of about half of all human genes (Bird, 1986). Methylation of cytosine within 5' CGIs is associated with loss of gene expression and has been seen in physiological conditions such as X chromosome inactivation and genomic imprinting (reviewed in Latham, 1996). Aberrant methylation of CGIs has been detected in genetic diseases

such as the fragile-X syndrome (Hansen *et al.*, 1992), in aging cells (Issa *et al.*, 1994) and in neoplasia. About half of the tumor suppressor genes which have been shown to be mutated in the germline of patients with familial cancer syndromes have also been shown to be aberrantly methylated in some
5 proportion of sporadic cancers, including *Rb*, *VHL*, *p16*, *hMLH1*, and *BRCA1* (reviewed in Baylin *et al.*, 1998; Jones 1997). TSG methylation in cancer is usually associated with (1) lack of gene transcription and (2) absence of coding region mutation. Thus it has been proposed that CGI methylation serves as an alternative mechanism of gene inactivation in cancer.

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The causes and global patterns of CGI methylation in human cancers remain poorly defined. Aging could play a factor in this process because methylation of several CGIs could be detected in an age-related manner in normal colon mucosa as well as in CRC (Issa *et al.*, 1994). In addition,
15 aberrant methylation of CGIs has been associated with the MI phenotype in CRC (Ahuja *et al.*, 1997) as well as specific carcinogen exposures (Issa *et al.*, 1996). However, an understanding of aberrant methylation in CRC has been somewhat limited by the small number of CGIs analyzed to date. In fact, previous studies have suggested that large numbers of CGIs are methylated in
20 immortalized cell lines (Antequera *et al.*, 1990), and it is not well understood whether this global aberrant methylation is caused by the cell culture conditions or whether they are an integral part of the pathogenesis of cancer.

Most of the methods developed to date for detection of methylated
25 cytosine depend upon cleavage of the phosphodiester bond alongside cytosine residues, using either methylation-sensitive restriction enzymes or reactive chemicals such as hydrazine which differentiate between cytosine and its 5-methyl derivative. Genomic sequencing protocols which identify a 5-MeC

residue in genomic DNA as a site that is not cleaved by any of the Maxam
Gilbert sequencing reactions have also been used, but still suffer
disadvantages such as the requirement for large amount of genomic DNA and
the difficulty in detecting a gap in a sequencing ladder which may contain
5 bands of varying intensity.

Mapping of methylated regions in DNA has relied primarily on
Southern hybridization approaches, based on the inability of methylation-
sensitive restriction enzymes to cleave sequences which contain one or more
10 methylated CpG sites. This method provides an assessment of the overall
methylation status of CpG islands, including some quantitative analysis, but is
relatively insensitive and requires large amounts of high molecular weight
DNA.

15 Another method utilizes bisulfite treatment of DNA to convert all
unmethylated cytosines to uracil. The altered DNA is amplified and
sequenced to show the methylation status of all CpG sites. However, this
method is technically difficult, labor intensive and without cloning amplified
products, it is less sensitive than Southern analysis, requiring approximately
20 10% of the alleles to be methylated for detection.

Identification of the earliest genetic changes in tumorigenesis is a
major focus in molecular cancer research. Diagnostic approaches based on
identification of these changes are likely to allow implementation of early
25 detection strategies and novel therapeutic approaches targeting these early
changes might lead to more effective cancer treatment.

SUMMARY OF THE INVENTION

The invention provides a method for detecting a methylated CpG-containing nucleic acid. This method can be used to identify sequences which are differentially methylated during a disease process such as a cell proliferative disorder.

In one embodiment, a method is provided for identifying a methylated CpG-containing nucleic acid. The method includes contacting a nucleic acid sample suspected of containing a CpG-containing nucleic acid, with a methylation sensitive restriction endonuclease that cleaves only unmethylated CpG sites, under conditions and for a time to allow cleavage of unmethylated nucleic acid; and contacting the sample with an isoschizomer of the methylation sensitive restriction endonuclease, wherein the isoschizomer of the methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG sites. Oligonucleotides are added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by the restriction endonuclease and the digested nucleic acid is amplified for further analysis.

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In another embodiment, a method is provided for detecting an age-associated disorder associated with methylation of CpG islands in a nucleic acid sequence of interest in a subject having or at risk of having said disorder. The method includes contacting a nucleic acid sample suspected of comprising a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease that cleaves only unmethylated CpG sites under conditions and for a time to allow cleavage of unmethylated nucleic acid, and contacting the sample with an isoschizomer of the methylation sensitive restriction

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endonuclease, wherein the isoschizomer of the methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG sites. Oligonucleotides are added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by the restriction endonuclease, and the digested nucleic acid is amplified. The amplified, digested nucleic acid is contacted with a membrane and the membrane is hybridized with a probe of interest.

In yet another embodiment, a method is provided for evaluating the response of a cell to an agent. The method includes contacting a nucleic acid sample suspected of containing a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease that cleaves only unmethylated CpG sites, under conditions and for a time to allow cleavage of unmethylated nucleic acid, and contacting the sample with an isoschizomer of the methylation sensitive restriction endonuclease, wherein the isoschizomer of the methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG sites. Oligonucleotides are added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by the restriction endonuclease, and the digested nucleic acid is amplified. The amplified, digested nucleic acid is adhered to a membrane and the membrane is hybridized with a probe of interest.

In a further embodiment, a kit for the detection of a methylated CpG-containing nucleic acid is provided. In one embodiment the kit includes a carrier means containing one or more containers including a container containing an oligonucleotide for ligation of the oligonucleotides to nucleic acid, a second container containing a methylation sensitive restriction

endonuclease and a third container containing an isoschizomer of the methylation sensitive endonuclease. In another embodiment the kit includes a carrier means containing one or more containers containing a membrane, wherein the membrane has a member of the group consisting SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 (MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33 immobilized on the membrane.

In a further embodiment, an isolated nucleic acid including a member selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 (MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33) is provided. An isolated methylated nucleic acid sequence having a sequence as set forth in a member of the group consisting of SEQ ID NOs:1-33 (MINT1-33) is also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram of MCA. A hypothetical fragment of genomic DNA is represented by a solid line, with 7 SmaI sites depicted by tick marks. Methylated SmaI sites are indicated by an m. Fragments B and D are CpG islands. B is methylated in both normal (right) and cancer (left), while D is differentially methylated in cancer. For MCA, unmethylated SmaI sites are eliminated by digestion with SmaI (which is methylation-sensitive and does not cleave when its recognition sequence CCCGGG contains a methylated CpG), which leaves the fragment blunt ended. Methylated SmaI sites are then digested with the non-methylation sensitive SmaI isoschizomer XmaI, which digests methylated CCCGGG sites, leaving a CCGG overhang (sticky ends). Adaptors are ligated to these sticky ends, and PCR is performed to amplify the methylated sequences. The MCA amplicons can be used directly in a dot blot analysis to study the methylation status of any gene for which a probe is available (left). Alternatively, MCA products can be used to clone differentially methylated sequence by RDA (right).

FIG. 2 shows the nucleotide sequence of a differentially Methylated Clone, MINT2 obtained by MCA Followed by RDA. The restriction endonuclease sites for SmaI are underlined. Primer sequences used for bisulfite-PCR are also underlined. The restriction endonuclease site for BstUI used to detect methylation after bisulfite PCR is shown by a gray box.

FIG. 3 show a map of the *versican* gene first exon (filled box) and flanking regions. The position of MINT11 is shown by a solid line (on top). CpG sites are indicated below. Location of the primers used for bisulfite-PCR are shown by arrows.

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FIG. 4 is a pictorial representation of global hypermethylation in CRC. Each column represents a separate gene locus. Each row is a primary colorectal cancer (samples above the bold solid line) or polyp (below the bold solid line). Black squares: methylation > 10%. Gray squares: 1-10% methylation. White squares: < 1% methylation. A: GH+MI+, B: GH+MI-, C: GH-MI+, D: GH-MI-, E: GH+, F: GH-. A-D are cancers. E and F are adenomas. MI denotes the presence of microsatellite instability. ND, not done.

FIG. 5 shows a model integrating CGI methylation in colorectal carcinogenesis.

FIGS. 6A-H are the nucleic acid sequences of MINT1-33 (SEQ ID NO: 1-33).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a method for identifying a methylated CpG-containing nucleic acid called methylated CpG island amplification (MCA). MCA can be used to study methylation in normal and neoplastic cells, and allows rapid screening of nucleic acid samples for the presence of hypermethylation of specific genes. MCA can also be used to clone genes and nucleic acid sequences differentially methylated in normal and abnormal tissues and cells.

It should be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the restriction enzyme" includes

reference to one or more restriction enzymes and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

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All publications mentioned herein are incorporated herein by reference in full for the purpose of describing and disclosing the methodologies which are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

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Any nucleic acid sample, in purified or nonpurified form, can be utilized as the starting nucleic acid or acids, provided it contains, or is suspected of containing, a nucleic acid sequence containing the target locus (e.g., CpG-containing nucleic acid). In general the CpG-containing nucleic acid will be DNA. However, the process may employ, for example, samples that contain DNA, or DNA and RNA, including messenger RNA, wherein DNA or RNA may be single stranded or double stranded, or a DNA-RNA hybrid may be included in the sample. A mixture of nucleic acids may also be employed. The specific nucleic acid sequence to be detected may be a fraction

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of a larger molecule or can be present initially as a discrete molecule, so that the specific sequence constitutes the entire nucleic acid. It is not necessary that the sequence to be studied be present initially in a pure form; the nucleic acid may be a minor fraction of a complex mixture, such as contained in
5 whole human DNA. The nucleic acid may be contained in a biological sample. Such samples include but are not limited to a serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, stool, or biopsy sample. The nucleic acid-containing sample used for detection of methylated CpG may be from any source including, but not limited to, brain, colon,
10 urogenital, hematopoietic, thymus, testis, ovarian, uterine, prostate, breast, colon, lung and renal tissue and may be extracted by a variety of techniques such as that described by Maniatis, *et al.* (Molecular Cloning: a Laboratory Manual, Cold Spring Harbor, NY, pp 280, 281, 1982).

15 The nucleic acid of interest can be any nucleic acid where it is desirable to detect the presence of a CpG island. In one embodiment, the CpG island comprises a CpG island located in a gene. A "CpG island" is a CpG rich region of a nucleic acid sequence. The nucleic acid sequence may be, for example, a p16, a Rb, a VHL, a hMLH1, or a BRCA1 gene. Alternatively the
20 nucleic acid of interest can be, for example, a MINT1-33 nucleic acid sequence. However, any gene or nucleic acid sequence of interest containing a CpG sequence can be detected using the method of the invention.

The presence of methylated CpG in the nucleic acid-containing
25 specimen may be indicative of a disorder. In one embodiment, the disorder is a cell proliferative disorder. A "cell proliferative disorder" is any disorder in which the proliferative capabilities of the affected cells is different from the normal proliferative capabilities of unaffected cells. An example of a cell

proliferative disorder is neoplasia. Malignant cells (*i.e.*, cancer) develop as a result of a multistep process. Specific, non-limiting examples of disorders associated with increased methylation of CpG-islands are colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer,
5 astrocytoma, glioblastoma, and neuroblastoma.

In another embodiment, the disorder is an age-associated disorder. The term "age-associated disorder" is used to describe a disorder observed with the biological progression of events occurring over time in a subject. Preferably,
10 the subject is a human. Non-limiting examples of age-associated disorders include, but are not limited to, atherosclerosis, diabetes melitis, and dementia. An age-associated disorder may also be a cell proliferative disorder. Examples of age-associated disorders which are cell proliferative disorders include colon cancer, lung cancer, breast cancer, prostate cancer, and
15 melanoma, amongst others. An age-associated disorder is further intended to mean the biological progression of events that occur during a disease process that affects the body, which mimic or substantially mimic all or part of the aging events which occur in a normal subject, but which occur in the diseased state over a shorter period of time.

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In one embodiment, the age-associated disorder is a "memory disorders or learning disorders" which are characterized by a statistically significant decrease in memory or learning assessed over time by the Randt Memory Test (Randt *et al.*, *Clin. Neuropsychol.*, 2:184, 1980), Wechsler
25 Memory Scale (*J. Psych.*, 19:87-95, 1945), Forward Digit Span test (Craik, *Age Differences in Human Memory*, in: Handbook of the Psychology of Aging, Birren, J., and Schaie, K., Eds., New York, Van Nostrand, 1977), Mini-Mental State Exam (Folstein *et al.*, *J. of Psych. Res.* 12:189-192, 1975),

or California Verbal Learning Test (CVLT) wherein such non-neurodegenerative pathological factors as aging, anxiety, fatigue, anger, depression, confusion, or vigor are controlled for. (See, U.S. Patent No. 5,063,206 for example).

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If the sample is impure (*e.g.*, plasma, serum, stool, ejaculate, sputum, saliva, cerebrospinal fluid or blood or a sample embedded in paraffin), it may be treated before amplification with a reagent effective for opening the cells, fluids, tissues, or animal cell membranes of the sample, and for exposing the nucleic acid(s). Methods for purifying or partially purifying nucleic acid from a sample are well known in the art (*e.g.*, Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Press, 1989, herein incorporated by reference).

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15 In one embodiment, a method is provided for identifying a methylated CpG-containing nucleic acid, including contacting a nucleic acid sample suspected of comprising a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease that cleaves only unmethylated CpG sites under conditions and for a time to allow cleavage of unmethylated nucleic acid. The sample is further contacted with an isoschizomer of the methylation sensitive restriction endonuclease, that cleaves both methylated and unmethylated CpG-sites, under conditions and for a time to allow cleavage of methylated nucleic acid. Oligonucleotides are added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by the restriction endonuclease, and the digested nucleic acid is amplified. Following identification, the methylated CpG-containing nucleic acid can be cloned, using method well

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known to one of skill in the art (see Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Press, 1989).

5 A "methylation sensitive restriction endonuclease" is a restriction endonuclease that includes CG as part of its recognition site and has altered activity when the C is methylated as compared to when the C is not methylated. Preferably, the methylation sensitive restriction endonuclease has inhibited activity when the C is methylated (*e.g.*, *SmaI*). Specific non-limiting examples of a methylation sensitive restriction endonucleases include *SmaI*,
10 *BssHII*, or *HpaII*. Such enzymes can be used alone or in combination. Other methylation sensitive restriction endonucleases will be known to those of skill in the art and include, but are not limited to *SacII*, *EagI*, and *BstUI*, for example. An "isoschizomer" of a methylation sensitive restriction endonuclease is a restriction endonuclease which recognizes the same
15 recognition site as a methylation sensitive restriction endonuclease but which cleaves both methylated and unmethylated CGs. One of skill in the art can readily determine appropriate conditions for a restriction endonuclease to cleave a nucleic acid (see Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Press, 1989). Without being bound by theory,
20 actively transcribed genes generally contain fewer methylated CGs than in other genes.

In the method of the invention, a nucleic acid of interest is cleaved with a methylation sensitive endonuclease. In one embodiment, cleavage with
25 the methylation sensitive endonuclease creates a sufficient overhang on the nucleic acid of interest. Following cleavage with the isoschizomer, the cleavage product can still have a sufficient overhang. An "overhang" refers to nucleic acid having two strands wherein the strands end in such a manner that

a few bases of one strand are not base paired to the other strand. A "sufficient overhang" refers to an overhang of sufficient length to allow specific hybridization of an oligonucleotide of interest. In one embodiment, a sufficient overhang is at least two bases in length. In another embodiment, the sufficient overhang is four or more bases in length. An overhang of a specific sequence on the nucleic acid of interest may be desired in order for an oligonucleotide of interest to hybridize. In this case, the isoschizomer can be used to create the overhang having the desired sequence on the nucleic acid of interest.

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In another embodiment, the cleavage with a methylation sensitive endonuclease results in a reaction product of the nucleic acid of interest that has a blunt end or an insufficient overhang. In this embodiment, an isoschizomer of the methylation sensitive restriction endonuclease can create a sufficient overhang on the nucleic acid of interest. "Blunt ends" refers to a flush ending of two strands, the sense strand and the antisense strand, of a nucleic acid.

15

Once a sufficient overhang is created on the nucleic acid of interest, an oligonucleotide is ligated to the nucleic acid cleaved of interest which has been cleaved by the methylation specific restriction endonuclease. "Ligation" is the attachment of two nucleic acid sequences by base pairing of substantially complementary sequences or by the formation of a covalent bonds between two nucleic acid sequences. An "oligonucleotide" is a nucleic acid sequence of 2 to 40 bases in length. Preferably the oligonucleotide is from 15 to 35 bases in length. In one embodiment, the oligonucleotide is ligated to the overhang on the nucleic acid sequence of interest by base pairing.

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In one embodiment, two oligonucleotides are utilized to form an adaptor. An "adaptor" is a double-stranded nucleic acid sequence with one end that has a sufficient single-stranded overhang at one or both ends such that the adaptor can be ligated by base-pairing to a sufficient overhang on a nucleic acid of interest that has been cleaved by a methylation sensitive restriction enzyme or an isoschizomer of a methylation sensitive restriction enzyme. In one embodiment, two oligonucleotides can be used to form an adaptor; these oligonucleotides are substantially complementary over their entire sequence except for the region(s) at the 5' and/or 3' ends that will form a single stranded overhang. The single stranded overhang is complementary to an overhang on the nucleic acid cleaved by a methylation sensitive restriction enzyme or an isoschizomer of a methylation sensitive restriction enzyme, such that the overhang on the nucleic acid of interest will base pair with the 3' or 5' single stranded end of the adaptor under appropriate conditions. The conditions will vary depending on the sequence composition (GC vs AT), the length, and the type of nucleic acid (see Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Plainview, NY, 1998).

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Following the ligation of the oligonucleotide, the nucleic acid of interest is amplified using a primer complementary to the oligonucleotide. Specifically, the term "primer" as used herein refers to a sequence comprising two or more deoxyribonucleotides or ribonucleotides, preferably more than three, and most preferably more than 8, which sequence is capable of initiating synthesis of a primer extension product, which is substantially complementary to a nucleic acid such as an adaptor or a ligated oligonucleotide. Environmental conditions conducive to synthesis include the presence of

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nucleoside triphosphates and an agent for polymerization, such as DNA polymerase, and a suitable temperature and pH. The primer is preferably single stranded for maximum efficiency in amplification, but may be double stranded. If double stranded, the primer is first treated to separate its strands
5 before being used to prepare extension products. In one embodiment, the primer is an oligodeoxyribo-nucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent for polymerization. The exact length of primer will depend on many factors, including temperature, buffer, and nucleotide composition. The
10 oligonucleotide primer typically contains 12-20 or more nucleotides, although it may contain fewer nucleotides.

Primers of the invention are designed to be "substantially" complementary to each strand of the oligonucleotide to be amplified and
15 include the appropriate G or C nucleotides as discussed above. This means that the primers must be sufficiently complementary to hybridize with their respective strands under conditions which allow the agent for polymerization to perform. In other words, the primers should have sufficient complementarity with a 5' and 3' oligonucleotide to hybridize therewith and
20 permit amplification of CpG containing nucleic acid sequence.

Primers of the invention are employed in the amplification process which is an enzymatic chain reaction that produces exponential quantities of target locus relative to the number of reaction steps involved (*e.g.*, polymerase
25 chain reaction or PCR). Typically, one primer is complementary to the negative (-) strand of the locus and the other is complementary to the positive (+) strand. Annealing the primers to denatured nucleic acid followed by extension with an enzyme, such as the large fragment of DNA Polymerase I

(Klenow) and nucleotides, results in newly synthesized + and - strands containing the target locus sequence. Because these newly synthesized sequences are also templates, repeated cycles of denaturing, primer annealing, and extension results in exponential production of the region (*i.e.*, the target locus sequence) defined by the primer. The product of the chain reaction is a discrete nucleic acid duplex with termini corresponding to the ends of the specific primers employed.

The oligonucleotide primers of the invention may be prepared using any suitable method, such as conventional phosphotriester and phosphodiester methods or automated embodiments thereof. In one such automated embodiment, diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage, *et al.* (*Tetrahedron Letters*, 22:1859-1862, 1981). One method for synthesizing oligonucleotides on a modified solid support is described in U.S. Patent No. 4,458,066.

Where the CpG-containing nucleic acid sequence of interest contains two strands, it is necessary to separate the strands of the nucleic acid before it can be used as a template for the amplification process. Strand separation can be effected either as a separate step or simultaneously with the synthesis of the primer extension products. This strand separation can be accomplished using various suitable denaturing conditions, including physical, chemical, or enzymatic means, the word "denaturing" includes all such means. One physical method of separating nucleic acid strands involves heating the nucleic acid until it is denatured. Typical heat denaturation may involve temperatures ranging from about 80° to 105°C for times ranging from about 1 to 10 minutes. Strand separation may also be induced by an enzyme from the class of enzymes known as helicases or by the enzyme RecA, which has helicase

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activity, and in the presence of riboATP, is known to denature DNA. The reaction conditions suitable for strand separation of nucleic acids with helicases are described by Kuhn Hoffmann-Berling (CSH-Quantitative Biology, 43:63, 1978) and techniques for using RecA are reviewed in
5 C. Radding (*Ann. Rev. Genetics*, 16:405-437, 1982).

When complementary strands of nucleic acid or acids are separated, regardless of whether the nucleic acid was originally double or single stranded, the separated strands are ready to be used as a template for the
10 synthesis of additional nucleic acid strands. This synthesis is performed under conditions allowing hybridization of primers to templates to occur. Generally synthesis occurs in a buffered aqueous solution, generally at a pH of about 7-9. Preferably, a molar excess (for genomic nucleic acid, usually about 10^8 :1 primer:template) of the two oligonucleotide primers is added to the buffer
15 containing the separated template strands. It is understood, however, that the amount of complementary strand may not be known if the process of the invention is used for diagnostic applications, so that the amount of primer relative to the amount of complementary strand cannot be determined with certainty. As a practical matter, however, the amount of primer added will
20 generally be in molar excess over the amount of complementary strand (template) when the sequence to be amplified is contained in a mixture of complicated long-chain nucleic acid strands. A large molar excess is preferred to improve the efficiency of the process.

25 The deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and dTTP are added to the synthesis mixture, either separately or together with the primers, in adequate amounts and the resulting solution is heated to about 90°-100°C from about 1 to 10 minutes, preferably from 1 to 4 minutes. After this

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heating period, the solution is allowed to cool to approximately room temperature, which is preferable for the primer hybridization. To the cooled mixture is added an appropriate agent for effecting the primer extension reaction (called herein "agent for polymerization"), and the reaction is allowed to occur under conditions known in the art. The agent for polymerization may also be added together with the other reagents if it is heat stable. This synthesis (or amplification) reaction may occur at room temperature up to a temperature above which the agent for polymerization no longer functions. Thus, for example, if DNA polymerase is used as the agent, the temperature is generally no greater than about 40°C. Most conveniently the reaction occurs at room temperature.

The agent for polymerization may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, *E. coli* DNA polymerase I, Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, other available DNA polymerases, polymerase muteins, reverse transcriptase, and other enzymes, including heat-stable enzymes (*i.e.*, those enzymes which perform primer extension after being subjected to temperatures sufficiently elevated to cause denaturation). Suitable enzymes will facilitate combination of the nucleotides in the proper manner to form the primer extension products which are complementary to each locus nucleic acid strand. Generally, the synthesis will be initiated at the 3' end of each primer and proceed in the 5' direction along the template strand, until synthesis terminates, producing molecules of different lengths. There may be agents for polymerization, however, which initiate synthesis at the 5' end and proceed in the other direction, using the same process as described above.

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Preferably, the method of amplifying is by PCR, as described herein and as is commonly used by those of ordinary skill in the art. However, alternative methods of amplification have been described and can also be employed.

5

Once amplified, the nucleic acid can be attached to a solid support, such as a membrane, and can be hybridized with any probe of interest, to detect any nucleic acid sequence. Several membranes are known to one of skill in the art for the adhesion of nucleic acid sequences. Specific non-
10 limiting examples of these membranes include nitrocellulose (Nitropure) or other membranes used in for detection of gene expression such as polyvinylchloride, diazotized paper and other commercially available membranes such as Genescreen™, Zetaprobe™ (Biorad), and Nytran™. Methods for attaching nucleic acids to these membranes are well known to one
15 of skill in the art. Alternatively, screening can be done in a liquid phase.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity,
20 nucleotide sequence composition (*e.g.*, GC v. AT content), and nucleic acid type (*e.g.*, RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

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An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency

conditions); and 0.1 x SSC at about 68°C (high stringency conditions).

Washing can be carried out using only one of these conditions, *e.g.*, high stringency conditions, or each of the conditions can be used, *e.g.*, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed.

- 5 However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically. In general, conditions of high stringency are used for the hybridization of the probe of interest.

- 10 The probe of interest can be detectably labeled, for example, with a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme. Those of ordinary skill in the art will know of other suitable labels for binding to the probe, or will be able to ascertain such, using routine experimentation.

15

- In one embodiment, representational difference analysis (RDA, see Lisitsyn *et al.*, *Science* 259:946-951, 1993, herein incorporated by reference) can be performed on CpG-containing nucleic acid following MCA. MCA utilizes kinetic and subtractive enrichment to purify restriction endonuclease
- 20 fragments present in one population of nucleic acid fragments but not in another. Thus, RDA enables the identification of small differences between the sequences of two nucleic acid populations. RDA uses nucleic acid from one population as a "tester" and nucleic acid from a second population as a "driver," in order to clone probes for single copy sequences present in (or
- 25 absent from) one of the two populations. In one embodiment, nucleic acid from a "normal" individual or sample, not having a disorder such as a cell-proliferative disorder is used as a "driver," and nucleic acid from an "affected" individual or sample, having the disorder such as a cell proliferative disorder

is used as a "tester." In one embodiment, the nucleic acid used as a "tester" is isolated from an individual having a cell proliferative disorder such as colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma. The nucleic acid used as a "driver" is thus normal colon, normal lung, normal kidney, normal blood cells, normal breast, normal prostate, normal uterus, normal astrocytes, normal glial and normal neurons, respectively. In an additional embodiment, the nucleic acid used as a "driver" is isolated from an individual having a cell proliferative disorder such as colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma. The nucleic acid used as a "tester" is thus normal colon, normal lung, normal kidney, normal blood cells, normal breast, normal prostate, normal uterus, normal astrocytes, normal glial and normal neurons, respectively. One of skill in the art will readily be able to identify the "tester" nucleic acid useful with to identify methylated nucleic acid sequences in given "driver" population.

SCREENING AGENTS FOR AN EFFECT ON METHYLATION

The invention provides a method for identifying an agent which can affect methylation. An agent can affect methylation by either increasing or decreasing methylation. The method includes incubating an agent and a sample containing a CpG-containing polynucleotide under conditions sufficient to allow the components to interact, and measuring the effect of the compound on the methylation of the CpG-containing nucleic acid. In one embodiment, the sample is a cell expressing a polynucleotide of interest. In another embodiment, the sample is substantially purified nucleic acid. "Substantially purified" nucleic acid is nucleic acid which has been separated from the cellular components which naturally accompany it, or from contaminating elements such as proteins, lipids, or chemical resins. Substantially pure nucleic acid can be extracted from any cell type, or can be chemically synthesized. Purity can be measured by any appropriate method, such as measuring the absorbance of light (e.g., A_{260}/A_{280} ratio).

The nucleic acid can be identified by the methylated CpG island amplification, as described above. The methylation of the polynucleotide in the sample can then be compared to the methylation of a control sample not incubated with the agent. The effect of the agent on methylation of a polynucleotide can be measured by assessing the methylation of the polynucleotide by the methods of the invention. Alternatively, the effect of the agent on methylation of a polynucleotide can be measured by assessing the expression of the polynucleotide of interest. Means of measuring expression are well known to one of skill in the art (e.g., Northern blotting or RNA dot blotting, amongst others).

The agents which affect methylation can include peptides, peptidomimetics, polypeptides, pharmaceuticals, and chemical compounds and biological agents. Psychotropic, antiviral, and chemotherapeutic compounds can also be tested using the method of the invention.

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“Incubating” includes conditions which allow contact between the test agent and the cell of interest. “Contacting” includes in solution and solid phase. The test agent may also be a combinatorial library for screening a plurality of compounds. Agents identified in the method of the invention can be further cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the isolation of a specific DNA sequence. Molecular techniques for DNA analysis (Landegren *et al.*, *Science* 242:229-237, 1988) and cloning have been reviewed (Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Plainview, NY, 1998).

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The sample can be any sample of interest. The sample may be a cell sample or a membrane sample prepared from a cell sample. Suitable cells include any host cells containing a nucleic acid including a CpG island. The cells can be primary cells or cells of a cell line.

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In one embodiment, the agent is incubated with the sample of interest suspected of including a CpG-containing nucleic acid and methylation is evaluated by MCA. Thus, nucleic acid from the sample suspected of including a CpG-containing nucleic acid is contacted with a methylation sensitive restriction endonuclease which cleaves only unmethylated CpG sites under conditions and for a time to allow cleavage of unmethylated nucleic acid. An isoschizomer of the methylation sensitive restriction endonuclease is

also utilized. An oligonucleotide is added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotide to nucleic acid cleaved by said restriction endonuclease, and the digested nucleic acid is amplified. The digested nucleic acid is adhered to a membrane, and the
5 membrane is hybridized with a probe of interest. In one embodiment, representation difference analysis can also be performed.

KITS

10 The materials for use in the assay of the invention are ideally suited for the preparation of a kit. Such a kit may comprise a carrier means containing one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. One of the container means can comprise a container containing an
15 oligonucleotide for ligation to nucleic acid cleaved by a methylation sensitive restriction endonuclease. One or more container means can also be included comprising a primer complementary to the oligonucleotide. In addition, one or more container means can also be included which comprise a methylation sensitive restriction endonuclease. One or more container means can also be
20 included containing an isoschizomer of said methylation sensitive restriction enzyme.

In another embodiment, the kit may comprise a carrier means containing one or more container means comprising a solid support, wherein
25 the solid support has a nucleic acid sequence selected from the group consisting of MINT1-33 immobilized on the solid support. In one embodiment, the solid support is a membrane. Several membranes are known to one of skill in the art for the adhesion of nucleic acid sequences. Specific

non-limiting examples of these membranes include nitrocellulose (Nitropure) or other membranes used in for detection of gene expression such as polyvinylchloride, diazotized paper and other commercially available membranes such as Genescreen™, Zetaprobe™ (Biorad), and Nytran™. The
5 MINT1-33 sequences immobilized on the solid support can then be hybridized to nucleic acid sequences produced by performing the MCA procedure on the nucleic acids of a sample of interest in order to determine if the nucleic acid sequences contained in the sample are methylated.

10 **POLYNUCLEOTIDES AND POLYPEPTIDES**

In another embodiment, the invention provides isolated MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotides (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively). These polynucleotides include DNA, cDNA and RNA
15 sequences which encode MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polypeptides. It is understood that naturally occurring, synthetic, and intentionally manipulated polynucleotides are included. For example,
20 MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 nucleic acids may be subjected to site-directed mutagenesis. The nucleic acid sequence for MINT1,

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MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 also includes antisense sequences, and sequences encoding dominant negative forms of MINT1, MINT2,
5 MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33.

The invention provides methylated and unmethylated forms of MINT1,
10 MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotides (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID
15 NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively). Methylated nucleic acid sequences are also provided which include MINT3, MINT5, MINT 7, MINT11, MINT12, MINT13, MINT16, MINT18, MINT21, MINT25, MINT26, MINT28, and MINT29
20 (SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, and SEQ ID NO:29, respectively). It is understood that naturally occurring, synthetic, and intentionally manipulated polynucleotides are included.

25

The polynucleotides of the invention includes "degenerate variants" sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon.

Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of a polypeptide encoded by the nucleotide sequence of SEQ ID NOs: 1-33 is functionally unchanged.

5 Specifically disclosed herein are methylated and unmethylated isolated polynucleotide sequences of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33. Preferably, the nucleotide sequence is SEQ ID NO:1, SEQ ID
10 NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively. Specifically disclosed herein are methylated isolated
15 polynucleotide sequences of MINT3, MINT5, MINT 7, MINT11, MINT12, MINT13, MINT16, MINT18, MINT21, MINT25, MINT26, MINT28, and MINT29. Preferably, the nucleotide sequence is SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:26,
20 SEQ ID NO:28, and SEQ ID NO:29, respectively. The term "polynucleotide" or "nucleic acid sequence" refers to a polymeric form of nucleotides at least 10 bases in length. By "isolated polynucleotide" is meant a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in
25 the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a

separate molecule (*e.g.*, a cDNA) independent of other sequences. The nucleotides of the invention can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of DNA.

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The polynucleotide encoding MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 includes SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID
10 NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, dominant negative forms of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14,
15 MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33, and nucleic acid sequences complementary to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20,
20 SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxynucleotides A, G, C, and T of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ
25 ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 are replaced by ribonucleotides A, G, C, and U, respectively. Also included in

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the invention are fragments of the above-described nucleic acid sequences that are and are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA that encoded by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 under physiological conditions or a close family member of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33. The term "selectively hybridize" refers to hybridization under moderately or highly stringent conditions which excludes non-related nucleotide sequences. Hybridization conditions have been described above.

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The MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33 nucleotide sequence includes the disclosed sequence and conservative variations of the polypeptides encoded by MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotides. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for

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asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

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MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 nucleic acid sequences can be expressed *in vitro* by DNA transfer into a suitable host cell.

10 "Host cells" are cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is
15 used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In one aspect, the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22,
20 MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotide sequences may be inserted into an expression vector. The term "expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the sequence of interest genetic sequences. Polynucleotide sequence which encode sequence
25 of interest can be operatively linked to expression control sequences.

"Operatively linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. An expression control sequence operatively linked to a coding

sequence is ligated such that expression of the coding sequence is achieved under conditions compatible with the expression control sequences. As used herein, the term "expression control sequences" refers to nucleic acid sequences that regulate the expression of a nucleic acid sequence to which it is
5 operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (*i.e.*, ATG) in
10 front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example,
15 leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements
20 which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters, are included in the invention (see, *e.g.*, Bitter *et al.*, *Methods in Enzymology* 153:516-544, 1987).
25 For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage γ , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from

mammalian viruses (*e.g.*, the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences of the invention.

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In the present invention, the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotide sequence may be inserted into an expression vector which contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg *et al.*, *Gene* 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.* 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (*e.g.*, T7, metallothionein I, or polyhedron promoters).

20

MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotide sequences can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and

25

plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

5 By "transformation" is meant a genetic change induced in a cell following incorporation of new DNA (*i.e.*, DNA exogenous to the cell). Where the cell is a mammalian cell, the genetic change is generally achieved by introduction of the DNA into the genome of the cell (*i.e.*, stable).

10 By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding sequence of interest. Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as
15 *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl_2 method using procedures well known in the art. Alternatively, MgCl_2 or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

20 When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be
25 cotransformed with DNA sequences encoding the sequence of interest, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to

transiently infect or transform eukaryotic cells and express the protein (see for example, Eukaryotic Viral Vectors, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

5 Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

10 In one embodiment, the invention provides substantially purified polypeptide encoded by MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotide sequences. The term "substantially purified" as used herein
15 refers to a polypeptide which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify a polypeptide encoded by MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30,
20 MINT31, MINT32 and MINT33 polynucleotide sequence using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24,
25 MINT27, MINT30, MINT31, MINT32 and MINT33 polypeptide can also be determined by amino-terminal amino acid sequence analysis.

Minor modifications of the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33 primary amino acid sequences may result in proteins which have substantially equivalent activity as compared to the unmodified counterpart polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity still exists.

The polypeptides of the invention also include dominant negative forms of the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polypeptide which do not have the biological activity of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polynucleotide sequence. A "dominant negative form" of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, or MINT33 is a polypeptide that is structurally similar to MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polypeptide but does not have wild-type MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 function. For example, a dominant-negative MINT1, MINT2,

MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polypeptide may interfere with wild-type MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, 5 MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 function by binding to, or otherwise sequestering, regulating agents, such as upstream or downstream components, that normally interact functionally with the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, 10 MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polypeptide.

EXAMPLES

The following examples are intended to illustrate but not to limit the 15 invention in any manner, shape, or form, either explicitly or implicitly. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLE 1

20 DETECTION OF METHYLATED CPG ISLANDS USING MCA

The principle underlying MCA involves amplification of closely spaced methylated SmaI sites to enrich for methylated CGIs. The MCA technique is outlined in Figure 1A. About 70 to 80% of CpG islands contain at least two closely spaced (<1kb) SmaI sites (CCCGGG). Only those SmaI 25 sites within these short distances can be amplified using MCA, ensuring representation of the most CpG rich sequences. Briefly, DNA is digested with SmaI, which cleaves only unmethylated sites, leaving blunt ends between the C and G. DNA is then digested with the SmaI isoschizomer XmaI, which

does cleave methylated CCCGGG sites, and which leaves a 4 base overhang. Adaptors are ligated to this overhang, and PCR is performed using primers complementary to these adaptors. The amplified DNA is then spotted on a nylon membrane and can be hybridized with any probe of interest.

5

As a model experiment, amplification of the *p16* gene CGI was examined because (1) hypermethylation of this CGI in cancer is well characterized, and correlates with silencing of the gene (Herman *et al.*, 1995), and (2) this CGI contains two closely spaced SmaI sites (400bp) which can be amplified by MCA. Initially, the reaction was optimized by testing different primers with a variable GC content, and different PCR conditions. As shown in Figure 1B, using primers with a 70% GC content, the *p16* CGI is amplified strongly in the Caco2 cell line, where it is known to be hypermethylated, while no signal above background was detected from any normal colon mucosa. To examine the quantitative aspect of MCA, DNA from Caco2 and normal colon mucosa were mixed in various proportions, and the methylation level of each mix was determined using MCA. MCA detected *p16* methylation in a semi-quantitative manner between 1% and 100% methylated alleles. Finally, MCA was performed on 109 samples of normal colonic mucosa and adjacent primary colorectal tumor that had previously been typed for *p16* methylation by Southern blot analysis (Ahuja *et al.*, 1997). MCA and Southern blot were concordant in 107/109 (98%) of the cases. In one case, MCA detected a low level of methylation (5-10 %) in a cancer sample that had been judged negative by Southern blot. In the other discordant case (positive by MCA, negative by Southern blot), the discordance may be related to heterogeneous *p16* methylation, as has been described (Costello *et al.*, 1996).

25

MCA is a novel PCR-based technique that allows for the rapid enrichment of hypermethylated CG rich sequences, with a high representation of methylated CpG islands. This technique can have several potential applications. MCA is very useful for the determination of the methylation status of a large number of samples at multiple loci simultaneously. By optimizing the PCR conditions, it should be readily adaptable to the study of the methylation status of any gene that has two closely spaced SmaI sites. As shown herein, there is a very high concordance rate between MCA and other methods for the detection of hypermethylation such as Southern blot analysis and bisulfite-based methods. However, MCA (1) requires good quality DNA, excluding the study of paraffin-embedded samples, (2) examines only a limited number of CpG sites within a CGI and (3) is sensitive to incomplete digestion using the methylation-sensitive enzyme SmaI. Nevertheless, many steps in MCA are amenable to automation and, by allowing for the examination of multiple genes relatively quickly, may have important applications in population-based studies of CGI methylation.

EXAMPLE 2

IDENTIFICATION OF DIFFERENTIALLY METHYLATED CG IN CRC BY MCA/ RDA

To identify novel CGIs aberrantly methylated in CRC, RDA (Lisitsyn *et al.*, 1993) was performed on MCA amplicons from the colon cancer cell line Caco2 as a tester, and a mixture of DNA from the normal colon mucosa of 5 different men (to avoid cloning polymorphic SmaI sites or inactive and methylated X chromosome genes from women) as a driver. Two separate experiments were conducted, one using a lower annealing temperature (72/C), and the other using a higher annealing temperature (77/C) and more GC rich primers. After two rounds of RDA, the PCR products were cloned, and

colonies containing inserts were identified by PCR. Based on initial experiments, we expected most of the recovered clones to contain Alu repetitive sequences, which are CG rich and hypermethylated (Kochanek *et al.*, 1993). All clones were therefore probed with an Alu fragment, and only non-hybridizing clones were analyzed further. Out of 160 non-Alu clones, 46 were independent clones and 33 of these (MINT1-33, Methylated in Tumors, SEQ ID NOs:1-33, respectively) appeared to be differentially methylated in Caco2 cells by comparing hybridization to MCA products from Caco2 and normal colon (Figure 1C). 19 of the clones (MINT1-19) were obtained using the lower annealing temperature, and 14 (MINT 20-33) using the higher temperature.

To confirm the aberrant methylation of these clones, Southern blot analysis was performed using DNA digested with SmaI or XmaI. All of the 33 clones were hypermethylated in Caco2 compared to normal colon mucosa. Of these 33, one clone (MINT13) detected highly repeated sequences and two clones (MINT18 and MINT28) appeared to correspond to mildly repeated gene families (data not shown). All others appeared to detect single copy DNA fragments. In addition, hypermethylation at CpG sites within the clones and distinct from the SmaI sites was confirmed by bisulfite-PCR for 6 clones. In each case, Caco2 was found to be hypermethylated at these sites.

By DNA sequencing (example shown in Figure 2), we found that 29 clones had a GC content greater than 50%, and satisfied the minimal criteria for CGIs (200bp, GC content>50%, CpG/GpC>0.5) (Gardiner-Garden and Frommer, 1987). As might be expected, clones obtained with the higher annealing temperature and more GC rich primers had a relatively higher GC content (Table 1). The size of each clone, percentage of GC nucleotide,

observed/expected CGs, sequence homology and, chromosomal location are summarized in Table 1. MINT5, MINT8, MINT11, MINT14 and MINT16 contained GC rich regions only in one end of the clones, and these may have been recovered from the edge of CGIs.

Table 1: Summary of the 33 Differentially Methylated Clones Isolated by MCA-RDA

Clone	Size (bp)	%GC	O/E	CGI	Blast Homology	Chromosome Map	Methylation Pattern
MINT1	528	56	0.6	Yes	None	5q13-14	Type C
MINT2	562	50	0.8	Yes	None	2p22-21	Type C
MINT3	563	55	1	Yes	Human EST AA557808	1p34-35	Type a
MINT4	481	60	0.8	Yes	None	15q25-26	Type a
MINT5	852	46	0.5	Yes*	Human CpG clone 88c1	14q21-22	Type a
MINT6	401	59	0.6	Yes	None	12q14-15	Type a
MINT7	481	49	0.9	Yes	Human genomic DNA	6p21-22	Type A
MINT8	617	46	0.5	Yes*	None	N.D	Type A
MINT9	605	54	0.3	No	None	1p34-35	Type A
MINT10	608	49	0.6	Yes	None	9q34-Ter	Type A
MINT11	637	49	0.6	Yes*	Versican	5q12-13	Type A
MINT12	552	49	0.6	Yes	CpG clone 33h2	7q31-32	Type C
MINT13	308	60	0.9	Yes	LINE1	N.D	Cell line
MINT14	620	54	0.4	Yes*	None	10p13-15	Type A
MINT15	641	53	0.7	Yes	None	11p12-13	Type A
MINT16	664	62	0.5	Yes*	Alpha-tubulin	2q	Type A
MINT17	491	54	0.7	Yes	None	6	Type C
MINT18	435	58	0.1	No	Acrogranin	N.D	Cell line
MINT19	443	55	0.2	No	None	N.D	Type A
MINT20	510	67	0.8	Yes	mouse OTP	N.D	Type A
MINT21	411	62	0.4	No	None	22q13	Type A
MINT22	438	60	0.9	Yes	None	10p12	Type A
MINT23	346	64	0.8	Yes	Csx	5q34-35	Type A
MINT24	525	63	0.7	Yes	None	3p25-26	Type A
MINT25	339	60	0.7	Yes	Human genomic DNA	22q11	Type C
MINT26	591	58	0.8	Yes	CpG clone 73e1	7q11	Type A
MINT27	242	74	0.7	Yes	None	N.D	Type C
MINT28	463	58	1	Yes	Ribosomal RNA gene	N.D	Type A
MINT29	429	60	0.7	Yes	CpG clone 20b1	7q11	N.D
MINT30	536	65	0.5	Yes	None	20q11	Type A
MINT31	673	65	0.8	Yes	None	17q21	Type C
MINT32	464	66	1	Yes	None	20q13	Type A
MINT33	139	65	0.8	Yes	None	N.D	N.D

O/E : Observed/expected numbers of CpGs. N.D : not determined.

* Only one portion of the clones has a CpG island.

By DNA homology search using the BLAST program (BLAST 2.0, default parameters, see <http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast?J-form=0>), 4 clones were identical to human gene sequences, four clones were identical to CGIs randomly sequenced from a CGI library (Cross *et al.*, 1994), one was identical to an EST, two clones were identical to high throughput genomic sequences deposited in Genbank, three clones had significant homology to other genes and the other 19 had no significant match in the database; MINT11 was identical to exon 1 and intron 1 of the human *versican* gene (Zimmerman *et al.*, 1989), and corresponded to the 3' edge of a promoter associated CGI; MINT14 was identical to exon 1 of the human *alpha-tubulin* gene (Dobner, P.R., *et al.*, 1987), and was also the 3' edge of the CGI; MINT 24 corresponded to the 3' noncoding region of the human homeobox gene *CSX* (Turbay *et al.*, 1996); MINT21 had a region with 94% homology at the nucleotide level to exon 2 of the mouse *OPT* gene (Simeone *et al.*, 1994) and probably represents the human homologue of this gene; MINT28 was homologous to ribosomal gene sequences; MINT18 was homologous to the acrogranin gene family. To examine the presence of potential promoter sequences in these clones, promoter prediction was performed using several computer programs (see programs available at <http://dot.imgen.bcm.tmc.edu:9331/seq-search/gene-search.html>). Twenty out of the 33 clones were predicted as promoters using the NNPP program, and 6 were predicted as promoters by using the TSSG program.

The chromosomal position of most of the unknown clones was determined using a somatic cell hybrid panel and a radiation hybrid panel (Table 1). Of note, MINT3 and MINT9 mapped to chromosome 1p35-36, MINT13 mapped to 7q31, MINT24 mapped to 3p25-26, MINT25 mapped to

22q11-Ter, and MINT31 mapped to 17q21. All of these chromosomal segments are areas that are frequently deleted in various tumors.

An important application of MCA is in the discovery of novel genes
5 hypermethylated in cancer. As demonstrated here, MCA coupled with RDA is
a rapid and powerful technology for this purpose, and compares favorably
with other described techniques (Hayashizaki *et al.*, 1994, Gonzalgo *et al.*,
1997; Huang *et al.*, 1997). In addition to the identification of genes
hypermethylated in cancer, MCA could potentially be used to discover novel
10 imprinted genes using parthenogenetic DNA (Kaneko-Ishino *et al.*, 1995), as
well as novel X-chromosome genes.

EXAMPLE 3

SILENCING OF THE *VERSICAN* GENE IN CRC

To determine whether some of these clones truly represented genes
silenced by methylation, we examined the *versican* gene in more detail.
Versican is a secreted glycoprotein that appears to be regulated by the *Rb*
tumor suppressor gene (Rohde *et al.*, 1996). MINT11 corresponds to part of
20 exon 1 and part of intron 1 of the *versican* gene (Figure 3A). Hypermethyla-
tion of the two *Sma*I sites in exon 1 and intron 1 in colon cancer cell lines was
confirmed by both Southern blot analysis and MCA. In order to determine if
this methylation was representative of the entire CGI, including the proximal
promoter, PCR was performed on bisulfite-treated DNA using primers
25 designed to amplify the region around the transcription start site of this gene.
The PCR product was then digested with restriction enzymes that distinguish
methylated from unmethylated DNA. The *versican* promoter was found to be
completely methylated in the colon cancer cell lines, DLD1, LOVO, SW48

and SW837, and partially methylated in HCT116 and HT29 (Figure 3B). In primary colon tumors, *versican* was hypermethylated in 17 out of 25 cases (68%). Interestingly, some methylation of the *versican* promoter was also found in normal tissues, albeit at lower levels when compared to tumors. The
5 level of methylation in normal colon mucosa increased with age of the patient (Fig3C), from an average of 6.9% in patients between 20 and 30 years of age, to an average of 28.9% in patients over 80. A linear regression analysis revealed a significant association between age and *versican* promoter methylation ($R=0.7$, $P<0.000001$). Using RT-PCR, we next examined the
10 expression of *versican* in normal colon mucosa and CRC cell lines. *Versican* was found to be expressed in normal colon epithelium, but was markedly down-regulated or absent in methylated colon cancer cell lines. Expression of *versican* in all these cell lines was easily restored after treatment with the demethylating agent, 5-aza-deoxycytidine. These data suggest that *versican*
15 becomes methylated in normal colon in an age-dependent manner, and that this leads to hypermethylation and loss of expression in most colorectal tumors.

Using MCA/RDA 33 differentially methylated clones were identified
20 and characterized in detail. By sequencing, we found that 29 out of the 33 clones satisfy the criteria of CpG islands, demonstrating that MCA can represent CGIs specifically. Of these 29 clones 5 were already known genes (*versican*, *alpha-tubulin*, *CSX*, *OPT* homologue and ribosomal RNA gene). Of these, *versican* is most interesting in that this proteoglycan is an Rb
25 inducible gene (Rohde *et al.*, 1996), suggesting that down regulation of this gene product may have an important role in colorectal carcinogenesis, where Rb mutations are rare. The data clearly show that aberrant methylation of the *versican* gene promoter is correlated with silencing of this gene. In addition,

methylation of the alpha-tubulin gene in Caco2 is consistent with the results of studying the gene expression profile of colorectal cancers using SAGE (Zang *et al.*, 1997), which demonstrated that alpha-tubulin is markedly down-regulated in CRC. Methylation of the CSX and OPT genes does not coincide with their 5' end, and is therefore not expected to silence these genes. It is possible, however, that these CpG islands are associated with alternate transcripts of the genes, or with other nearby genes, which would then be silenced by methylation (Wutz, A., *et al.*, 1997). Finally, methylation of ribosomal genes has previously been seen in aging tissues (Swisshelm, K., *et al.*, 1990) and therefore is not surprising to find in cancers. Because some of the clones recovered are in the exon 1 region of expressed genes, identification of new tumor suppressor genes might be facilitated by using MCA/RDA clones as probes for screening cDNA library. Indeed, based on their chromosome location, several clones map to chromosomal regions thought to harbor TSGs because they are highly deleted in various tumors (*e.g.*, chromosome 1p35, 3p25-26, 7q31, 17q21 and 22q11-Ter).

EXAMPLE 4

TWO TYPES OF METHYLATION IN CRC

By examining the methylation status of several known genes in colorectal tumors, it has been previously demonstrated that some genes tend to be methylated in an age-dependent manner in normal colon (Issa *et al.*, 1994), and are frequently methylated in CRC, while others are methylated in cancers exclusively (Ahuja *et al.*, 1997). To examine this issue on a genome wide level in some detail, the methylation profile of 31 MINT clones in a panel of colorectal tumors and corresponding normal colon mucosa was examined using MCA (two clones could not be accurately studied because of high background (MINT29) or small size (MINT33)). Because all of these clones

were recovered from a CRC cell line, there was an initial concern that many of these were not representative of methylation in primary (uncultured) tumors. However, of the 31 clones, 29 were also found to be methylated in some primary CRC. The two clones methylated only in the cell line Caco2 were (1) MINT14, a LINE element, and (2) MINT18, a sequence that had a very low CpG frequency and did not qualify as a CGI. Thus, all non-repetitive CGIs recovered were methylated in primary CRC as well as cell lines. Hypermethylation patterns of these 29 clones fell into two distinct categories. A majority of the clones (22 out of 29) were found to be frequently methylated (>70%) in the tumors tested, and a slight amount of methylation was also detected in normal colon mucosa. For all of these clones, the normal colon mucosa obtained from young patients showed less methylation compared to the normal mucosa from older patients (Figure 4B). Thus, the majority of CGIs hypermethylated in CRC are methylated in normal colon mucosa as well, in an age related manner. This methylation was named Type A for aging-specific methylation.

The remaining 7 clones were methylated exclusively in CRC, and their frequency of methylation was significantly lower than type A methylation (ranging from 10% to 50%). This type of methylation was named type C for cancer-specific.

Recently, several reports have suggested that aberrant methylation of CGIs may play an important role in cancer development (Baylin *et al.*, 1998; Jones 1997). However, there is little integrated information on aberrant CGI methylation in cancer at multiple loci, probably because of the lack of a
5 method to detect methylation in a large number of samples for unselected CGIs throughout the genome. Furthermore, it has been shown that cultured cell lines have a high degree of CGI methylation (Antequera *et al.*, 1990) but it was not known to what extent this reflects methylation in primary cancers. To address these issues, the relatively quantitative and high output features of
10 MCA allowed us to determine the methylation profile of 31 differentially methylated loci in a panel of colorectal carcinomas.

Despite the fact that all sequences were initially recovered from a colon cancer cell line, only 2 out of the 31 clones showed cell line restricted
15 methylation. From the sequence data, one of these two clones was a repeated sequences (LINE1), and the other was not a CGI. Thus most of the single copy clones recovered proved to be methylated not only in cell lines but also in some primary colon cancers. Analysis of these 29 clones revealed two distinct types of hypermethylation in cancer (Type A for aging and Type C for
20 cancer), which may have distinct causes, and different roles in cancer development. Type A methylation was seen in the majority of these clones: 22 of 29 (74%) clones were methylated in an age-related manner in normal colon tissue, and hypermethylated at a high frequency in CRC, as we have shown for the ER gene (Issa *et al.*, 1994) and others (Issa *et al.*, 1996; Ahuja *et al.*, submitted). These results suggest that a large number of CGIs in the
25 human genome are incrementally methylated during the aging process and, for many genes, this methylation correlates with reduced gene expression as shown for ER (Issa *et al.*, 1994) and versican. Although the mechanism of

Type A methylation is unknown, it is likely to result from physiological processes rather than a genetic alteration because (1) it is very frequent and affects large numbers of cells, (2) it is present in all individuals, not just patients with cancer and (3) this process is gene and tissue specific (Ahuja *et al.*, submitted). Because the methylation status at a given CGI is thought to be related to positive (methylator) factors (Mummaneni *et al.*, 1993; Mummaneni *et al.*, 1995; Magewu and Jones, 1994, Vertino *et al.*, 1996) and negative (protector) factors (Macleod *et al.*, 1994; Brandeis *et al.*, 1994; Turker and Bestor, 1997; Chen *et al.*, 1997), it is possible that for some genes, this balance favors slightly de-novo methylation, and that this is reflected by progressive hypermethylation after repeated cell divisions.

EXAMPLE 5

GLOBAL HYPERMETHYLATION IN CRC

15

To understand the patterns of cancer-specific methylation in CRC, the methylation status of all 7 type C clones was analyzed, as well as *p16* in primary cancers and polyps (Figure 4). Two of these clones (MINT1 and MINT2) were studied by both MCA and bisulfite-PCR, and the concordance between the two techniques was found to be 98%. *P16* was studied by both MCA and Southern blot, with a concordance rate of 98%. When we considered the six clones that were methylated in more than 10% of the cases, as well as *p16*, a remarkable pattern emerged (summarized in Figure 5 and Table 2).

20

Table 2: Percentage of Tumors Methylated at Multiple MINT Loci, *p16* and *hMLH1*.

	Type C Methylation										Type A Methylation									
	MINT	MINT	MINT	MINT	MINT	MINT	MINT	<i>p16</i>	<i>hMLH</i>		MINT	MINT	MINT	MINT	MINT	MINT	MINT	MINT	MINT	MINT
	1	2	12	17	25	31			1		6	8	22	24	30	32				
MI+ Cancer																				
EI+ (n=12)	100%	100%	100%	92%	100%	92%	83%	100%			100%	100%	100%	100%	100%	100%				
EI- (n=4)	25%	0%	0%	0%	0%	0%	0%	25%			100%	100%	100%	75%	75%	50%				
MI- Cancer																				
EI+ (n=17)	65%	82%	59%	41%	100%	82%	59%	0%			94%	100%	100%	100%	60%	93%				
EI- (n=17)	0%	12%	0%	0%	12%	0%	0%	0%			82%	86%	100%	82%	40%	65%				
Adenoma																				
EI+ (n=7)	86%	86%	71%	29%	86%	71%	57%	0%			86%	83%	100%	100%	43%	86%				
EI- (n=8)	0%	0%	0%	0%	0%	13%	0%	0%			50%	100%	86%	50%	13%	25%				

The 50 CRC fell into two distinct groups: (1) A group with a high level of Type C methylation, whereby all the tumors had methylation of 4 or more loci simultaneously and (2) a group where methylation of any type C clone is extremely rare. Thus, the first group of tumors appears to display profound global hypermethylation (GH+), which is lacking in the second group (GH-). Interestingly, there was a great concordance between methylation of the *p16* gene, which was not selected for by our cloning process, and the presence of GH. In sharp contrast, Type A methylation was not significantly different between GH+ and GH- tumors (Table 2).

GH was also detected in a subset of colorectal adenomas (Figure 5), suggesting that it is an early event in carcinogenesis. Interestingly, while 5 of 5 small adenomas (<7 mm) were GH-, 6 of 9 large adenomas (>10 mm) were GH+, suggesting that this defect may be acquired in the transition between small and large adenomas. In 6 cases, both an adenoma and a cancer from the same patients were examined. In one of these, GH was detected both in the adenoma and the cancer; in 3 cases, GH was detected in the cancer but not in the adenoma and in 2 cases, GH was detected in neither the adenoma nor the cancer.

By contrast to type A methylation, type C methylation is relatively infrequent in primary CRC, and is never observed in normal colon mucosa. Furthermore, detailed analysis of type C methylation in CRC revealed a striking pattern, suggesting the presence of global hypermethylation in a subset of these tumors: GH positive cases are characterized by frequent and concordant methylation of all type C clones examined, such that each tumor has at least four methylation events. By contrast, type C methylation is

virtually non-existent in tumors without GH. This concordance cannot be due to simple experimental variation or artifacts because (1) methylation was verified using separate methods (MCA, bisulfite-PCR and Southern blots), (2) the concordance was not limited to MCA/RDA derived clones since it also
5 affected the p16 (Herman *et al.*, 1995) and hMLH1 (Kane *et al.*, 1997) genes, and (3) there was no significant difference in type A methylation between GH+ and GH- tumors. Global hypermethylation appears to be an early event in the development of CRC, being detectable in large pre-neoplastic adenomas. Because many genes are potential candidates for inactivation
10 through promoter methylation (Baylin *et al.*, 1998; Jones, 1996), global hypermethylation may have profound pathophysiologic consequences in neoplasia through the simultaneous inactivation of tumor-suppressor genes (such as p16), metastasis-suppressor genes (such as E-cadherin), angiogenesis inhibitors (such as Thrombospondin-1) and others. In fact, our data suggest
15 that global hypermethylation could also result in mismatch repair deficiency through methylation and inactivation of the hMLH1 promoter, and may explain up to 75% of cases of sporadic CRC with microsatellite instability. The causes of type A and type C methylation are probably different because the latter is detected only in a limited number of cases, and the genes affected
20 are different. Because of the remarkable concordance in type C methylation among GH+ cases, it appears likely that these tumors all share a specific defect in the maintenance of the methylation-free state in CGIs. This defect could be either aberrant de-novo methylation (through a mutation in DNA-methyltransferase for example), or loss of protection against de-novo
25 methylation, through the loss of a trans-activating factor (Macleod *et al.*, 1994; Chen *et al.*, 1997). Because DNA-methyltransferase activity is similar in the two groups, the latter hypothesis is more likely. Thus, at least in colorectal cancer, it appears likely that type C methylation (an epigenetic error) is

actually caused by a genetic event that results in an increased chance of methylating a subset of CGIs. Ironically, this epigenetic defect may then result in additional genetic lesions through the induction of mismatch-repair deficiency.

5

EXAMPLE 6
MICROSATELLITE INSTABILITY IS LINKED TO
GLOBAL HYPERMETHYLATION IN CRC

10 In a previous study (Ahuja *et al.*, 1997), a link was reported between microsatellite instability and a hypermethylator phenotype in sporadic CRC. Relatively few mutations in mismatch repair genes have been reported in sporadic MI+ cancers, but *hMLH1* methylation has recently been observed in some cases (Kane *et al.*, 1997). To determine the relation between global
15 hypermethylation and microsatellite instability in CRC, we measured *hMLH1* methylation using bisulfite/PCR in our panel of CRC which had also been previously typed for the presence of microsatellite instability (Figure 5). *hMLH1* was studied by bisulfite-PCR only because it does not have 2 SmaI sites in its CGI. Overall, 16 out of 50 (32%) cancers had evidence of
20 microsatellite instability. Among the 29 GH+ cases, 12 had evidence of *hMLH1* methylation, suggesting that *hMLH1* is one of the targets of global hypermethylation in CRC. All of these 12 tumors had microsatellite instability. By contrast, *hMLH1* methylation was detected in only one of the
21 GH- cases. These data establish a strong link between the GH phenotype,
25 *hMLH1* methylation and microsatellite instability in CRC. Two lines of evidence suggest that microsatellite instability may follow, and be caused by, global hypermethylation and *hMLH1* methylation. First, GH is detectable in about half of colonic adenomas, but none of these tumors have *hMLH1*

methylation, and microsatellite instability is extremely rare in this pre-neoplastic lesion (Samowitz and Slattery, 1997). Second, GH is not simply caused by mismatch repair defects because microsatellite instability is absent in more than half of the GH+ cases, and GH was absent in 4 of the 16 cancers with microsatellite instability. Overall, our data suggest that, in sporadic CRC, the majority (12 out of 16, or 75%) of cases with microsatellite instability may be caused by GH followed by *hMLH1* methylation, loss of *hMLH1* expression and resultant mismatch repair deficiency (Herman *et al.*, submitted).

Based on these data, the following model has been developed integrating CGI methylation into CRC development (Figure 6). In this model, CGI methylation plays two distinct roles, and appears to arise through distinct mechanisms. Initially, type A methylation arises as a function of age in normal colorectal epithelial cells. By affecting genes that regulate the growth and/or differentiation of these cells, such methylation results in a hyperproliferative state, which is thought to precede tumor formation in the colon (reviewed by Lipkin, 1988). Such hyperproliferation is known to arise with age in colorectal epithelium (Holt *et al.*, 1988, Roncucci *et al.*, 1988), and to be marked in patients with CRC. The cause of type A methylation is unknown, but without being bound by theory it is possible that it is related to endogenous factors inherent to the structure of DNA, and that it may be modulated by factors such as level of ongoing expression and exposure to carcinogenic insults. Furthermore, modulation of type A methylation may provide one possible explanation for the reduction in CRC tumorigenesis by reducing levels of DNA-methyltransferase (Laird *et al.*, 1995).

A second major role for CGI methylation appears later, perhaps at the transition between small and large adenomas in the colon. This methylation (type C) affects only a subset of tumors, which then evolve along a pathway of global hypermethylation. This GH leads to cancer development through the simultaneous inactivation of multiple tumor-suppressor genes such as p16, and induction of mismatch repair deficiency through inactivation of hMLH1. The cause of this global hypermethylation is unknown, but may well be related to inactivation of a gene that protects CGIs from de-novo methylation. Finally, we propose that tumors without GH evolve along more classic genetic instability pathways, including chromosomal instability (Lengauer *et al.*, 1997A). Interestingly in this regard, Lengauer *et al.* found an inverse correlation between chromosomal instability and MMR deficiency in CRC cell lines (Lengauer *et al.*, 1997B).

While based on CRC, this model is applicable to most human malignancies. In evidence has also been found for type A and type C methylation in brain tumors (Li *et al.*, 1998). Preliminary evidence also suggests the presence of global hypermethylation in multiple types of cancers, including stomach cancers, brain tumors and hematopoietic malignancies.

In conclusion, a novel method, MCA, has been developed to selectively amplify methylated CGIs. Using MCA/RDA 33 differentially methylated clones in CRC were isolated. The methylation profile of these clones revealed that nearly all methylation in CRC can be accounted for by (1) age-related methylation and (2) a hypermethylator phenotype presumably caused by global hypermethylation. Deciphering the mechanisms underlying these phenomena should facilitate the early detection, prevention and therapy of cancers, including colorectal cancers.

EXAMPLE 7
IDENTIFICATION OF CACNA1G AS A TARGET FOR
HYPERMETHYLATION ON HUMAN CHROMOSOME 17q21

5

To identify genes differentially methylated in colorectal cancer, methylated CpG island amplification was used followed by representational difference analysis (Razin and Cedar, *Cell* 17: 473-476, 1994, herein incorporated by reference). One of the clones recovered (MINT31, see above) mapped to human chromosome 17q21 using a radiation hybrid panel, and a Blast search revealed this fragment to be completely identical to part of a BAC clone (Genbank: AC004590) sequenced by high throughput genomic sequence. The region surrounding MINT31 fulfills the criteria of a CpG island: GC content 0.67, CpG/GpC ratio 0.78 and a total of 305 CpG sites in a 4 kb region. Using this CpG island and 10 kb of flanking sequences in a Blast analysis, several regions highly homologous to the rat T-type calcium channel gene, *CACNA1G*, were identified (Perez-Reyes et al., *Nature* 391: 896-900, 1998, herein incorporated by reference). Several ESTs were also identified in this region. Using Genscan, 2 putative coding sequences (G1, and G2) were identified. Blastp analysis revealed that G1 has a high homology to the EH-domain-binding protein, epsin, while G2 is homologous to a C-elegans hypothetical protein (accession No. 2496828).

The MINT31 CpG island corresponds to the 3' regions of G1 and G2, based on the direction of the open reading frame and the presence of a poly A tail, and is unlikely to influence their transcription. The EST closest to MINT31 (H13333) was sequenced entirely and was found not to contain a continuous open reading frame, but a poly-adenylation signal was identified

on one end, along with a poly A tail. These data suggest that H13333 corresponds to the last 2 exons of an unidentified gene. MINT31 is in the intron of this gene and is unlikely to influence its transcription. However, based on both promoter prediction (TSSG) analysis of this region and
5 homology to the rat *CACNA1G* sequence, the MINT31 CpG island is also in the 5' region of human *CACNA1G* gene and may play a role in its transcriptional activity.

The human *CACNA1G* sequence deposited in Genbank lacks the 5'
10 region of the gene, when compared to the rat homologue. To determine the 5' region of human *CACNA1G*, we amplified cDNA by RT-PCR using primers based on the BAC sequence (Genbank: AC004590, herein incorporated by reference). The PCR products were cloned and sequenced, and the genomic organization of the gene was determined by comparing the newly identified
15 sequences as well as the known sequences to the BAC that covers this region. *CACNA1G* is composed of 34 exons which span a 70 kb area. Based on sequences deposited in Genbank, the gene has two possible 3' ends caused by alternate splicing. *CACNA1G* is highly homologous to rat *CACNA1G* with 93% identity at the protein level, and 89% identity at the nucleotide level. The
20 5' flanking region of *CACNA1G* lacks TATA and CAAT boxes, which is similar to many housekeeping genes. A putative TFIID binding site was identified 547-556 bp upstream from the translation start site, and several other potential transcription factor binding sites such as AP1 (1 site), AP2 (2 sites) and SP1 (10 sites), were identified upstream of *CACNA1G* exon 1 using
25 the promoter prediction program, TESS (data not shown).

The *CACNA1G* CpG island is 4 kb, and is larger than many typical CpG islands. MINT31 corresponds to the 5' edge of the island while

CACNA1G is in the 3' region. It is not known whether large CpG islands such as this are coordinately regulated with regards to protection from methylation, and aberrant methylation in cancer. To address this issue, the methylation status of the 5' region of *CACNA1G* was studied using bisulfite-PCR of DNA from normal tissues as well as 35 human cancer cell lines from colon, lung, prostate, breast and hematopoietic tumors. The CpG island was divided into 8 regions and their methylation status was examined separately. The genomic DNA was treated with sodium bisulfite and PCR amplified using primers containing no or a minimum number of CpG sites. Methylated alleles were detected by digesting the PCR products using restriction enzymes which specifically cleave sites created or retained due to the presence of methylated CpGs. None of the regions was methylated in normal colon, consistent with a uniform protection against de-novo methylation.

Regions 1 and 2 were frequently methylated in cancer cell lines, and behaved in a concordant manner. These 2 regions were methylated in most cancer cell types except gliomas, and most cell lines where methylation was found methylated both regions simultaneously. Region 3, which is less CG rich than any of the other regions, had either no methylation or very low levels of methylation in most cell lines. Regions 5, 6, and 7 behaved quite differently compared to 1-3. Methylation of these regions was less frequent than regions 1-2, as 22/35 cell lines had no detectable methylation there, despite often showing methylation of region 1-2. However, when methylation was present (in 13/35 cell lines), it affected all 3 regions simultaneously, although to varying extents. Finally, regions 4 and 8 behaved differentially again, being partially methylated primarily in colon and breast cell lines. Therefore, with regards to hypermethylation in cancer, the CpG rich region upstream of *CACNA1G* appears to be composed of 2 CpG islands which

behave independently. MINT31 corresponds to the upstream CpG island (island 1, regions 1 and 2), while the 5' region of *CACNA1G* is contained in the downstream CpG island (island 2, regions 5-7). Regions 3, 4 and 8 correspond to the edge of these CpG islands, and behave a little differentially than the hearts of the CpG islands, as previously described for the *E-Cad* gene (Graff, et al., *J. Biol. Chem.* 272: 22322-22329, 1997).

Overall, the methylation patterns fell into 5 distinct categories: (1) No methylation in any region (normal tissue). (2) Slight methylation of island 1 (6 cell lines, see for example TSU-PRL in Fig. 2). (3) Heavy methylation of island 1 but no methylation of island 2 (16 cell lines, see for example Caco2 in Fig. 2). (4) Heavy methylation of island 1 and moderate to heavy methylation of island 2 (6 cell lines, see for example RKO and Raji in Fig. 2). (5) High methylation of island 1 and low to moderate methylation of island 2 (7 cell lines, see for example MB-231 in Fig. 2).

In a previous study of rat *CACNA1G*, this gene was shown to be expressed most abundantly in the brain (Perez-Reyes et al., *Nature* 391: 896-900. 1998). To determine the expression of *CACNA1G* in normal and neoplastic human cells, RT-PCR was performed using cDNA from various normal tissues and from a panel of 27 tumor cell lines. *CACNA1G* was expressed ubiquitously in a variety of tissues and cell lines. In normal tissues expression was relatively low but easily detectable, while most cell lines had relatively high expression of *CACNA1G*. However, some cell lines had negligible or totally absent levels of *CACNA1G* expression. The results of *CACNA1G* expression was correlated with the detailed methylation analysis previously described. In this analysis, a remarkable pattern emerged. Methylation of region 1-4 and 8 had no effect on *CACNA1G* expression.

However, there was a strong correlation between methylation of regions 5-7 and expression of the gene. In fact, all cell lines that lack methylation of this region strongly express the gene. All 6 cell lines with pattern 4 methylation studied had no detectable expression. Finally, the 7 cell lines with pattern 5 methylation (examples DLD-1 and MB-453) had variable levels of expression ranging from very low to near normal. The fact that patterns 3 and 5 differ significantly with regards to expression, but are almost identical with regards to methylation of all regions except 7 suggests that this area is important in the inactivation of *CACNA1G*.

To confirm whether methylation of the 5' CpG island of *CACNA1G* is really associated with gene inactivation, 3 non-expressing cell lines showing pattern 4 methylation (RKO, SW48 and Raji) and 2 weakly expressing cell lines showing pattern 5 methylation (MB-231 and MB-435) were treated with 1 M of the methyl-transferase inhibitor 5-deoxy-azacitidine. After treatment, all these cell lines re-expressed *CACNA1G* mRNA. Consistent with re-expression, demethylation of region 7 was observed after 5-deoxy-azacitidine treatment (Fig. 3C).

De novo cytosine methylation is thought to sometimes occur in vitro during cell propagation (Antequera et al., *Cell* 62: 503-514, 1990). To determine whether the methylation of *CACNA1G* occurs in vivo, primary human tumors were examined for methylation of the 5' region of *CACNA1G*. Aberrant methylation was detected in 17 out of 49 (35%) colorectal cancers, 4 out of 28 colorectal adenomas (25%), 4 out of 16 (25%) gastric cancers and 3 out of 17 (18%) acute myelogenous leukemia cases. In colorectal cancers, there was a significant correlation between methylation of *CACNA1G* and methylation of *p16* ($p < 0.005$) and *hMLH1* ($p < 0.001$), as well as a strong

correlation with the presence of microsatellite instability, and the recently identified CpG island methylator phenotype (CIMP), supporting that *CACNA1G* is also a target for CIMP in colorectal cancer.

5 To determine whether aberrant methylation of the 5' region of *CACNA1G* affects the expression status of this gene in primary tumors, we performed RT-PCR using cDNA from a series of colorectal adenomas. Six out of 8 cases which showed no methylation of region 7 expressed *CACNA1G*. In sharp contrast, all 5 cases that showed methylation of region 7 had no
10 detectable expression of this gene.

 Thus, a human T-type calcium channel gene (*CACNA1G*) has been identified and cloned using the MINT31 sequence as a probe. The human T-type calcium channel gene has been determined to be a target of aberrant
15 methylation and silencing in human tumors. The data show that MINT31 (a representative sequence of MINT1-33) can be used as a probe to identify genes that play a role in disorders such as cell proliferative disorders.

 Detailed analysis of the CpG island upstream of *CACNA1G* revealed
20 that methylation 300 to 800 bp upstream of the gene closely correlated with transcriptional inactivation. The *CACNA1G* promoter is contained in a large CG rich area that is not coordinately methylated in cancer. The CpG island around MINT31 is much more frequently methylated in cancers compared to that just upstream of *CACNA1G*. This may simply be caused by differential
25 susceptibility to de-novo methylation between these two regions, with methylation of MINT31 serving as a trigger, and eventually spreading to *CACNA1G*, as described in other genes (Graff, et al., *J. Biol. Chem.* 272: 22322-22329, 1997). However, it is likely that these 2 regions are controlled

by different mechanisms because (1) cell lines kept in culture for countless generations do not in fact spread methylation from MINT31 to *CACNA1G* (e.g., Caco2), (2) region 3 that separates the 2 islands is infrequently and sparsely methylated in cancer and (3) 2 cases of primary colorectal cancer were found which are methylated at the *CACNA1G* promoter but not at MINT31). Therefore, methylation of MINT31 appears to be independent of methylation of *CACNA1G* suggesting that they are 2 distinct CpG islands regulated by different mechanisms. These data leave open the possibility that MINT31 is the promoter for an unidentified gene, which may perhaps be transcribed opposite to *CACNA1G*.

Many CpG islands of silenced genes appear to be methylated uniformly and heavily throughout the island (e.g., Graff, et al., *J. Biol. Chem.* 272: 22322-22329, 1997). In contrast the methylation patterns of the 5' region of *CACNA1G* (region 5-7) was heterogeneous in the cell lines which did not express this gene. Nevertheless, methylation does appear to play a role in *CACNA1G* repression since demethylation readily reactivates the gene.

The causes of *CACNA1G* methylation remain to be determined. Methylation was not detected in normal colon mucosa, placenta, normal breast epithelium and normal bone marrow, including samples from aged patients, suggesting that methylation of this region is cancer specific. However, there was a significant correlation between methylation of *CACNA1G* and other tumor suppressor genes such as *p16* and *hMLH1*. Thus, *CACNA1G* probably is a target for the recently described CIMP phenotype, which results in a form of epigenetic instability with simultaneous inactivation of multiple genes. It should be noted that a gene identified by the method of the invention

(MINT31) has been successfully utilized to identify another gene of interest (*CACNA1G*) whose methylation pattern correlates with the presence of specific cell proliferative disorders.

5 T-type calcium channels are involved not only in electrophysiological rhythm generation but also in the control of cytosolic calcium during cell proliferation and cell death (reviewed in Berridge, et al., *Nature* 395: 645-648, 1998). The results demonstrate that the expression of *CACNA1G* is not limited to brain and heart, suggesting that it may play a role in these other
10 tissues. It has previously been shown that Ca^{2+} influx via T-type channels is an important factor during the initial stages of cell death such as apoptosis (Berridge, et al., *Nature* 395: 645-648, 1998), ischemia (Fern, *J. Neurosci.* 18: 7232-7243, 1998) and complement-induced cytotoxicity (Newsholme, et al., *Biochem. J.* 295: 773-779, 1993.). These studies determining the methylation
15 status of the *CACNA1G* suggest that the impairment of voltage gated calcium channels may play an important role in cancer development and progression through altering calcium signaling.

EXAMPLE 8**EXPERIMENTAL PROCEDURES***Methylated CpG Island Amplification.*

The procedure is outlined in Figure 1. Five µg of DNA were digested
5 with 100 units of SmaI for 6 hours (all restriction enzymes were from NEB).
The DNA was then digested with 20 units of XmaI for 16 hours. DNA
fragments were then precipitated with ethanol. RXMA and RMCA PCR
adaptors were prepared by incubation of the oligonucleotides RXMA24
(5'-AGCACTCTCCAGCCTCTCACCGAC-3') (SEQ ID NO: 34) and
10 RXMA12 (5'-CCGGGTCGGTGA-3') (SEQ ID NO:35), or RMCA24
(5'-CCACCGCCATCCGAGCCTTTCTGC-3') (SEQ ID NO:36) and
RMCA12 (5'-CCGGGCAGAAAG-3') (SEQ ID NO:37) at 65°C for two min.
followed by cooling to room temperature. 0.5 µg of DNA was ligated to
0.5 nmol of RXMA or RMCA adaptor using T4 DNA ligase (NEB). PCR was
15 performed using 3 µl of each of the ligation mix as a template in a 100 µl
volume containing 100 pmol of RXA24 or RMC24 primer, 5 units of Taq
DNA polymerase, (GIBCO-BRL.), 4 mM MgCl₂, 16 mM of NH₄ (SO₄)₂,
10mg/ml of BSA, and 5% v/v DMSO. The reaction mixture was incubated at
72°C at 5 min and at 95°C for 3 min. Samples were then subjected to 25
20 cycles of amplification consisting of 1 min at 95°C, and 3 min either at 72°C
or 77°C in a thermal cycler (Hybaid, Inc.). The final extension time was 10
min.

Detection of Aberrant Methylation Using MCA.

25 MCA products from normal colon mucosa and corresponding cancer
tissues were prepared as described above. One µg of MCA products was
resuspended in 4 µl of TE (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0), mixed
with 2 µl of 20 X SSC, and 1 µl aliquot of this mix was blotted onto nylon

membranes (Nunc) using a 96 well replication system (Nunc). The membranes were baked at 80°C, UV crosslinked for 2 min. and hybridized using ³²P labeled probes. Each sample was blotted in duplicate. Each filter included mixtures of a positive control (Caco2) and a negative control (normal colon mucosa from an 18 year old individual). The filters were exposed to a phosphor screen for 24 to 72 hours and developed using a phosphorimager (Molecular Dynamics). The intensity of each signal was calculated using the Image Quant software, and methylation levels were determined relative to the control samples.

10

RDA.

RDA was performed essentially as previously reported (Lisitsyn *et al.*, 1993) with the following modifications. For the first and second rounds of competitive hybridization, 500 ng and 100 ng of ligation mix was used, respectively. To eliminate the digested adaptor, a cDNA spun column (Amersham) was used instead of excising from the agarose gel. Primers used for the first and second rounds of RDA are as follows :

JXMA24	5'-ACCGACGTCGACTATCCATGAACC-3'	SEQ ID NO:38
JXMA12	5'-CCGGGGTTCATG-3'	SEQ ID NO:39
JMCA24	5'-GTGAGGGTTCGGATCTGGCTGGCTC-3'	SEQ ID NO:40
JMCA12	5'-CCGGGAGCCAGC-3'	SEQ ID NO:41
NXMA24	5'-AGGCAACTGTGCTATCCGAGTGAC-3'	SEQ ID NO:42
NXMA12	5'-CCGGGTCACTCG-3'	SEQ ID NO:43
NMCA24	5'-GTTAGCGGACACAGGGCGGGTCAC-3'	SEQ ID NO:44
NMCA12	5'-CCGGGTGACCCG-3'	SEQ ID NO:45

After the second round of competitive hybridization, PCR products were digested with XmaI. The J adaptor was eliminated by column filtration. The PCR products were then subcloned into Bluescript SK(-) (Stratagene). To screen for inserts, a total of 396 clones were cultured overnight in LB medium with ampicillin and 3 µl of the culture was directly used as template for a PCR reaction. Each clone was amplified with

T3 (5'-AATTAACCCTCACTAAAGGG-3') (SEQ ID NO:46)

and

T7 (5'-GTAATACGACTCACTATAGGGC-3') (SEQ ID NO:47)

primers,

blotted onto nylon membranes, and screened for cross hybridization with 32P labeled inserts. The clones differentially hybridizing to tester and driver MCA products were further characterized by Southern blot analysis and DNA sequencing.

Southern blot analysis.

Five µg of DNA was digested with 20-100 units of restriction enzymes as specified by the manufacturer (NEB). DNA fragments were separated by agarose gel electrophoresis and transferred to a nylon membrane (Zeta-probe, Bio-Rad). Filters were hybridized with 32P-labeled probes and washed at 65°C with 2X SSC, 0.1 % SDS for 10 min. twice, and 0.1X SSC, 0.1 % SDS for 20 min. Filters were then exposed to a phosphor screen for 24-72 hours and analyzed by using a phosphorimager (Molecular Dynamics).

DNA sequencing and analysis.

Plasmid DNA was prepared using the Wizard Plus Minipreps (Promega) according to the suppliers recommendation. Sequence analysis was carried out at the Johns Hopkins Core Sequencing Facility using automated

DNA sequencers (Applied Biosystems). Sequence homologies were identified using the BLAST program of the National Center for Biotechnology Information (NCBI) available at <http://www.ncbi.nlm.nih.gov/BLAST> using the default parameters of the web site. Putative promoter sequences were
 5 predicted using the computer programs NNPP and TSSG available through the Baylor college of Medicine launcher at <http://dot.imgen.bcm.tmc.edu:9331>.

Bisulfite-restriction methylation analysis.

DNA from colon tumors, cell lines and normal colon mucosa was
 10 treated with bisulfite as reported previously (Herman *et al.*, 1996). Primers used for PCR were as follows:

15	hMLH1,	5'-TAGTAGTYGTTTTAGGGAGGGA-3' (SEQ ID NO:44), 5'-TCTAAATACTCAACRAAAATACCTT-3' (SEQ ID NO:45);
20	MINT1,	5'-GGGTTGGAGAGTAGGGGAGTT-3' (SEQ ID NO:46), 5'-CCATCTAAAATTACCTCRATAACTTA-3' (SEQ ID NO:47);
25	MINT2,	5'-YGTTATGATTTTGTGTTAGTTAAT-3' (SEQ ID NO:48), 5'-TACACCAACTACCCAACCTC-3' (SEQ ID NO:409);
	Versican,	5'-TTATTAYGTTTTTTATGTGATT-3' (V1) (SEQ ID NO:50), 5'-ACCTTCTACCAATTACTTCTTT-3' (V2) (SEQ ID NO:51).

Ten to 20 µl of the amplified products were digested with restriction enzymes which distinguish methylated from unmethylated sequences as

reported previously (Sadri *et al.*, 1996; Xiong *et al.*, 1997), electrophoresed on 3 % agarose or 5% acrylamide gels, and visualized by ethidium bromide staining.

5 *RT-PCR*

Total RNA was prepared from normal colon epithelium and tumor cell lines using TRIZOL (GIBCO-BRL). To study gene expression following demethylation, cell lines were treated with 1 M of 5-aza-2'-deoxycytidine for 2-5 days. cDNA was prepared using random hexamers and reverse transcriptase as specified by the manufacturer (Boehringer). The expression of versican was determined by RT-PCR using the primers

VF 5'-GCTGCCTATGAAGATGGATTTGAGC-3' (SEQ ID NO:52) and

VR 5'-GGAGTTCCCCCACTGT-TGCCA-3' (SEQ ID NO:53).

15

The PCR products were visualized by ethidium bromide staining. The cDNA samples were also amplified using GAPDH gene, primers

GAPF 5'-CGGAGTCAACGGATTGGTCGTAT-3' (SEQ ID NO:54) and

20

GAPR 5'-AGCCTTCTCCATGGTGGTGAAGAC-3' (SEQ ID NO:55)

as a control for RNA integrity. All reactions were performed using RT (-) controls where the reverse transcriptase enzyme was omitted.

25

Chromosomal mapping.

The chromosomal location of clones that did not correspond to known genes was determined using a human-rodent somatic cell hybrid panel and a

radiation hybrid panel (Research Genetics). PCR reactions were performed using 30 ng of each of the hybrid panel DNA as a template in a 40 μ l volume containing 15 pmol of each primer, 0.5 units of Taq DNA polymerase, (GIBCO BRL), 2mM MgCl₂, BSA and 5% DMSO. First denaturation was
5 carried out at 95°C for 3 min. Samples were then subjected to 35 cycles of amplification consisting of 25 sec. at 94°C, 1 min at 60 to 68°C and 1.5 min. at 72°C in a thermal cycler (Hybaid). The final extension time was 10 min. Ten μ l of the PCR product were electrophoresed in a 2 % agarose and the genotype of each panel was determined. Linkage analysis was performed
10 using the RH server of Stanford University as described (Stewart *et al.*, 1997).

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention.
15 Accordingly, the invention is limited only by the following claims.

-72-

What is claimed is:

1. A method for identifying a methylated CpG-containing nucleic acid, comprising
 - a) contacting a nucleic acid sample suspected of containing a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease, under conditions and for a time to allow cleavage of the nucleic acid;
 - b) contacting the sample with an isoschizomer of said methylation sensitive restriction endonuclease, wherein said isoschizomer of said methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG sites.
 - c) adding oligonucleotides to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to the nucleic acid cleaved by said restriction endonuclease; and
 - d) amplifying said cleaved nucleic acid.
2. The method of claim 1, wherein said methylation sensitive restriction endonuclease is SmaI.
3. The method of claim 1, wherein said amplifying is by polymerase chain reaction amplification.

4. The method of claim 3, wherein said amplifying by polymerase chain reaction amplification comprises annealing primers complementary to said oligonucleotide.
5. The method of claim 1, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:34 (RXMA24) and SEQ ID NO:35 (RXMA12).
6. The method of claim 1, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:36 (RMCA24) and SEQ ID NO:37 (RMCA12).
7. The method of claim 1, further comprising adhering the amplified nucleic acid to a membrane.
8. The method of claim 7, further comprising hybridizing the membrane with a probe of interest.
9. The method of claim 1, wherein the CpG containing nucleic acid comprises a methylated CpG island.
10. The method of claim 9, wherein the CpG island comprises a CpG island located in a gene selected from the group consisting of a p16, a Rb, a VHL, a hMLH1, and a BRCA1 gene.

11. The method of claim 1, wherein said sample is selected from the group consisting of a brain cell, a colon cell, a urogenital cell, a lung cell, a renal cell, a hematopoietic cell, a breast cell, a thymus cell, a testis cell, an ovarian cell, a uterine cell, an intestinal cell, serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, and stool.
12. The method of claim 1, wherein the presence of methylated CpG - containing nucleic acid in the sample is indicative of a cell proliferative disorder.
13. The method of claim 12, wherein the cell proliferative disorder is selected from the group consisting of colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma.
14. The method of claim 1, further comprising performing representation difference analysis, wherein said representation difference analysis comprises hybridizing a driving nucleic acid as a driver.
15. The method of claim 14, wherein said representation difference analysis uses nucleic acid isolated from a member of the group consisting of normal colon, normal lung, normal kidney, normal blood cells, normal breast, normal prostate, normal uterus, normal astrocytes, normal glial and normal neurons.
16. A nucleic acid identified by the method of claim 1.
17. A vector comprising the nucleic acid of claim 16.

18. A method for detecting an age-associated disorder, associated with methylation of CpG islands, in a nucleic acid sequence of interest in a subject having or at risk of having said disorder, comprising:
 - a) contacting a nucleic acid sample suspected of comprising a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease, under conditions and for a time to allow cleavage of the nucleic acid;
 - b) contacting the sample with an isoschizomer of said methylation sensitive restriction endonuclease, wherein said isoschizomer of said methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG-sites, under conditions and for a time to allow cleavage of methylated nucleic acid;
 - c) adding oligonucleotides to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by said restriction endonuclease;
 - d) amplifying said cleaved nucleic acid;
 - e) adhering the amplified digested nucleic acid to a membrane; and
 - f) hybridizing the membrane with a probe of interest.
19. The method of claim 18, wherein the sample is selected from the group consisting of brain cells, colon cells, urogenital cell, lung cells, renal cells, hematopoietic cells, breast cell, thymus cells, testis cells, ovarian cells, uterine cells, serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, and stool.
20. The method of claim 18, wherein the probe of interest is a nucleic acid sequence.

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21. The method of claim 18, wherein the nucleic acid sequence is selected from the group consisting of a p16, a Rb, a VHL, a hMLH1, and a BRCA1 nucleic acid.
22. The method of claim 21, wherein said nucleic acid sequence is a p16 nucleic acid sequence.
23. The method of claim 18, wherein the sample is a tissue sample or a biological fluid sample.
24. The method of claim 18, wherein the probe is detectably labeled.
25. The method of claim 24, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, and an enzyme.
26. The method of claim 18, wherein said age-associated disorder is selected from the group consisting of atherosclerosis, diabetes melitis, and dementia.
27. The method of claim 18, wherein said age-associated disorder is a cell proliferative disorder.
28. The method of claim 18, wherein the nucleic acid of interest is a member of the group consisting of SEQ ID NOs:1-33 (MINT 1-33).

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29. The method of claim 27, wherein said cell proliferative disorder is selected from the group consisting of colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma.
30. The method of claim 18, further comprising performing representation difference analysis, wherein said representation difference analysis comprises hybridizing a driving nucleic acid as a driver.
31. The method of claim 30, wherein said representation difference analysis uses nucleic acid isolated from a member of the group consisting of normal colon, normal lung, normal kidney, normal blood cells, normal breast, normal prostate, normal uterus, normal astrocytes, normal glial and normal neurons.

32. A method for determining the response of a cell to an agent, comprising:
- a) contacting a nucleic acid sample suspected of comprising a CpG-containing nucleic acid from said cell with a methylation sensitive restriction endonuclease, under conditions and for a time to allow cleavage of unmethylated nucleic acid;
 - b) contacting the sample with an isoschizomer of said methylation sensitive restriction endonuclease, wherein said isoschizomer of said methylation sensitive restriction endonuclease cleaves methylated and unmethylated CpG-sites, under conditions and for a time to allow cleavage of methylated nucleic acid;
 - c) adding an oligonucleotide to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotide to nucleic acid cleaved by said restriction endonuclease;
 - d) amplifying said cleaved nucleic acid;
 - e) adhering the amplified cleaved nucleic acid to a membrane; and
 - f) hybridizing the membrane with a probe of interest.
33. The method of claim 32, further comprising performing representation difference analysis, wherein said representation difference analysis comprises hybridizing a nucleic acid as a driver.

34. The method of claim 32, wherein the agent is selected from the group consisting of peptide, peptidomimetic, chemical compound, and a pharmaceutical compound.
35. The method of claim 32, wherein said agent is a chemotherapeutic agent.
36. The method of claim 32, wherein said methylation sensitive restriction endonuclease is SmaI.
37. The method of claim 32, wherein said amplifying is by polymerase chain reaction amplification.
38. The method of claim 37, wherein said amplifying by polymerase chain reaction amplification comprises annealing primers complementary to said oligonucleotide.
39. The method of claim 32, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:34 (RXMA24) and SEQ ID NO:35 (RXMA12).
40. The method of claim 32, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:36 (RMCA24) and SEQ ID NO:37 (RMCA12).

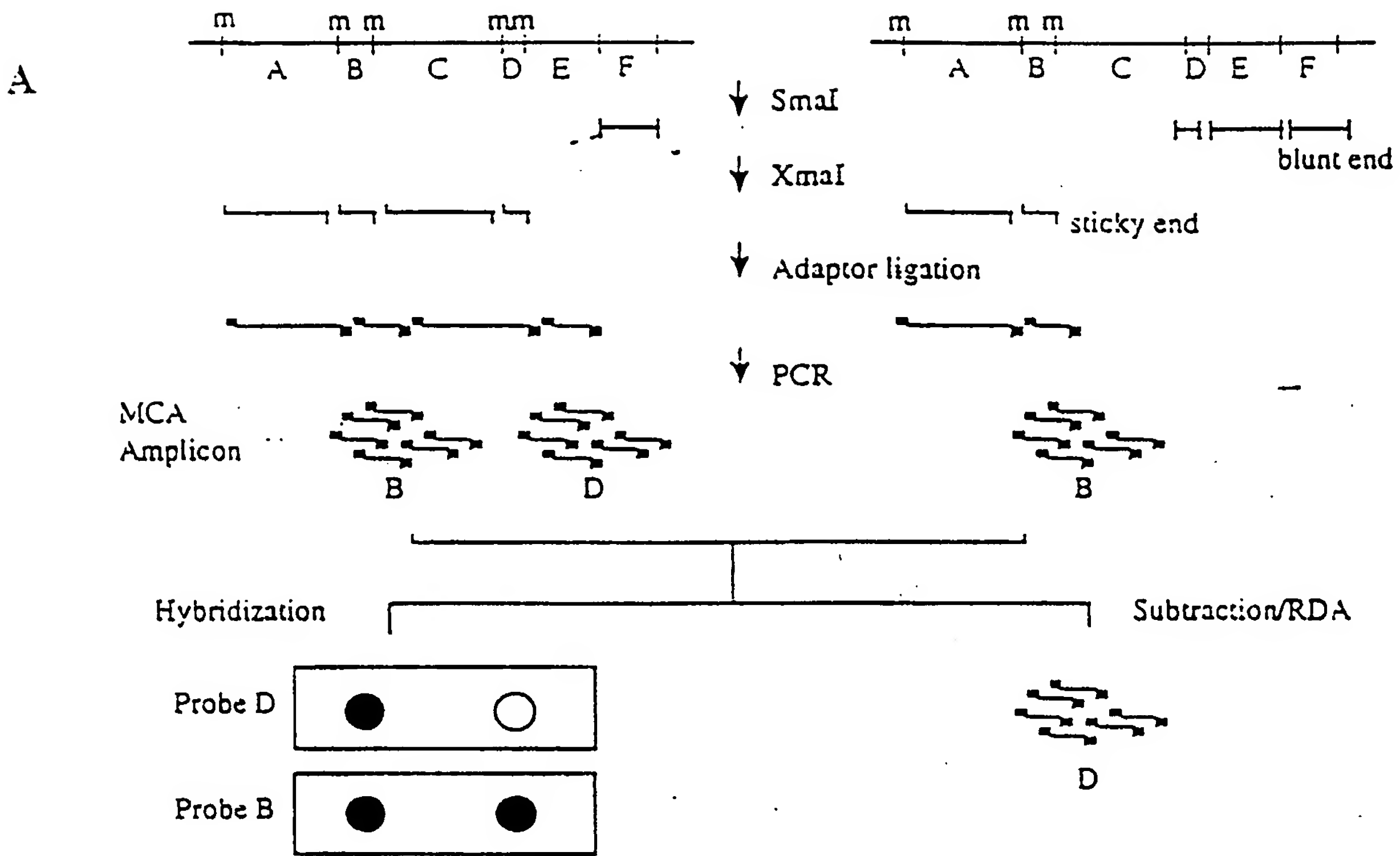
41. The method of claim 32, wherein said cell is selected from the group consisting of a brain cell, a colon cell, an intestinal cell, a urogenital cell, a lung cell, a renal cell, a hematopoietic cell, a breast cell, a thymus cell, a testis cell, an ovarian cell, a uterine cell, an exocrine cell, and an endocrine cell.
42. A kit useful for the detection of a methylated CpG-containing nucleic acid comprising carrier means containing one or more containers comprising a container containing oligonucleotides for ligation to nucleic acid, a second container containing a methylation sensitive restriction endonuclease and a third container containing an isoschizomer of the methylation sensitive restriction endonuclease.
43. The kit of claim 42, wherein said oligonucleotides comprises a sequence as set forth in a member of the group selected from SEQ ID NO:34 (RXMA24) and SEQ ID NO:35 (RXMA12).
44. The kit of claim 42, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:36 (RMCA24) and SEQ ID NO:37 (RMCA12).
45. The kit of claim 42, further comprising one or more containers comprising a primer complementary to said oligonucleotide.

46. A kit useful for the detection of a methylated CpG-containing nucleic acid comprising a carrier means containing one or more containers comprising a membrane, wherein said membrane has a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 (MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33 immobilized on said membrane.
47. An isolated nucleic acid comprising a member selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33 (MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33), and degenerate variants thereof.
48. The nucleic acid of claim 47, wherein said nucleic acid is methylated.

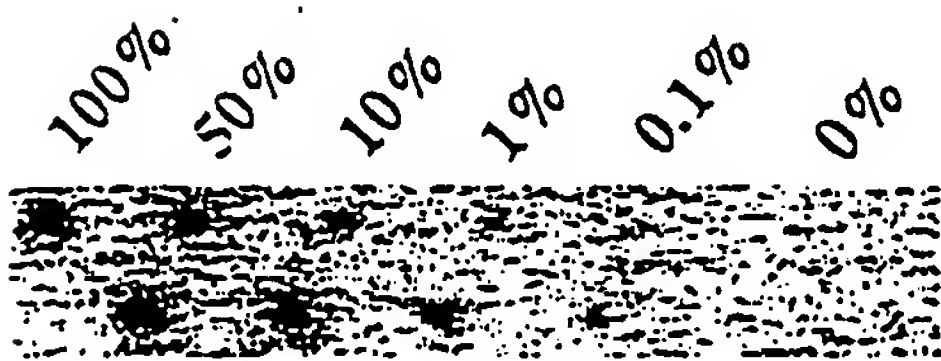
49. The nucleic acid of claim 48, wherein said nucleic acid is unmethylated.
50. An substantially purified polypeptide encoded by the nucleic acid of claim 47.
51. The nucleic acid of claim 47, wherein said nucleic acid is operatively linked to an expression control sequence.
52. The nucleic acid of claim 51, wherein the expression control sequence is a promoter.
53. The nucleic acid of claim 52, wherein the promoter is tissue specific.
54. An expression vector containing the nucleic acid of claim 47.
55. The vector of claim 54, wherein the vector is a plasmid.
56. The vector of claim 54, wherein the vector is a viral vector.
57. The vector of claim 56, wherein the viral vector is a retroviral vector.
58. A host cell containing the vector of claim 54.
59. The host cell of claim 58, wherein the cell is a eukaryotic cell.
60. The host cell of claim 58, wherein the cell is a prokaryotic cell.

61. An isolated nucleic acid sequence comprising a methylated nucleic acid having a sequence as set forth in a member of the group consisting of SEQ ID NOs:1-33.
62. A method of identifying a compound that affects methylation of a nucleic acid, comprising:
 - a) incubating components comprising the compound and a sample comprising a nucleic acid sequence identified by the method of claim 1 under conditions sufficient to allow the components to interact; and
 - b) determining the effect of the compound on expression of the nucleic acid sequence.
63. The method of claim 62, wherein said sample is a cell.
64. The method of claim 62, wherein said sample is a substantially purified nucleic acid.
65. The method of claim 62, wherein the compound is selected from the group consisting of a peptide, a peptidomimetic, a chemical compound, and a pharmaceutical compound.

Figure 1



B



C

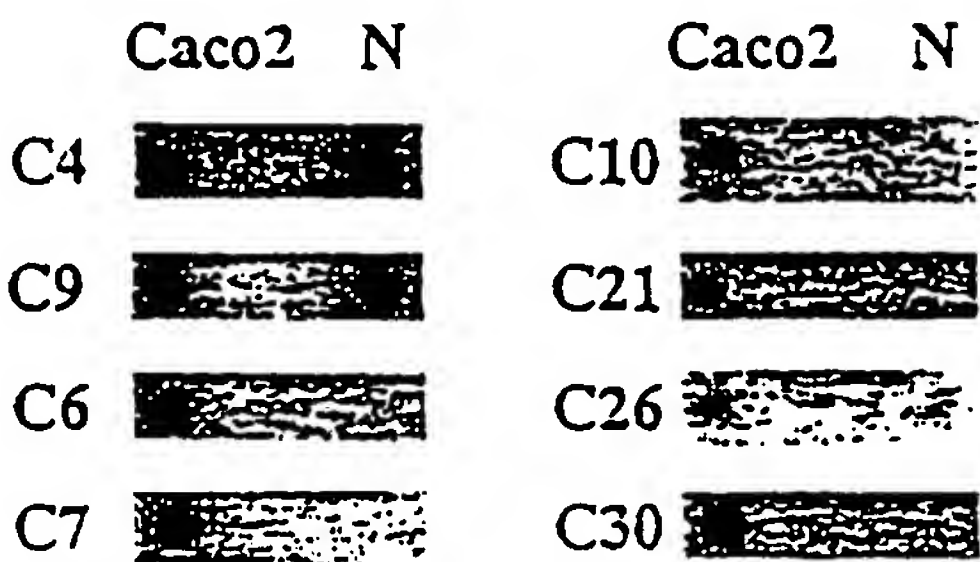
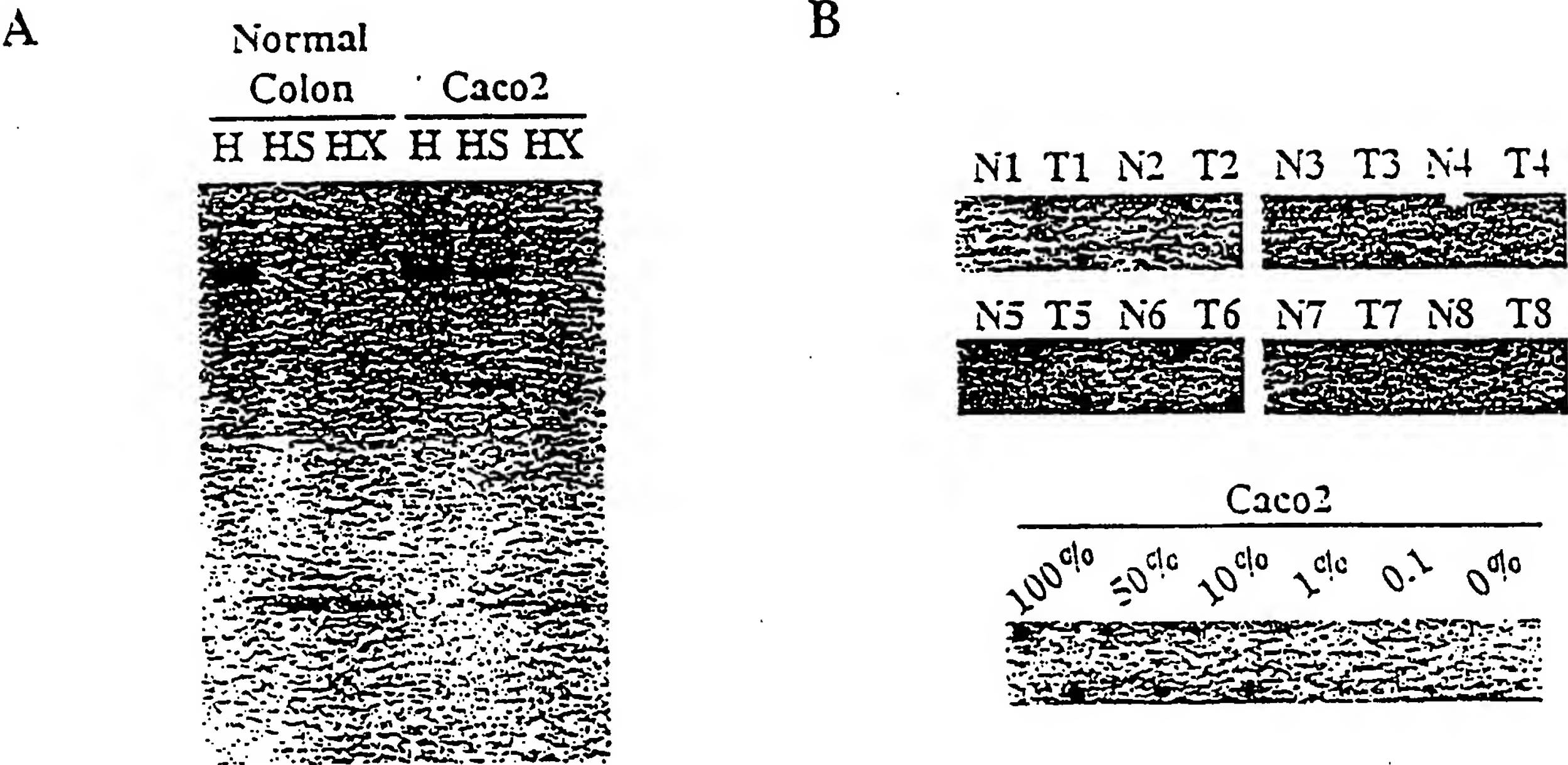
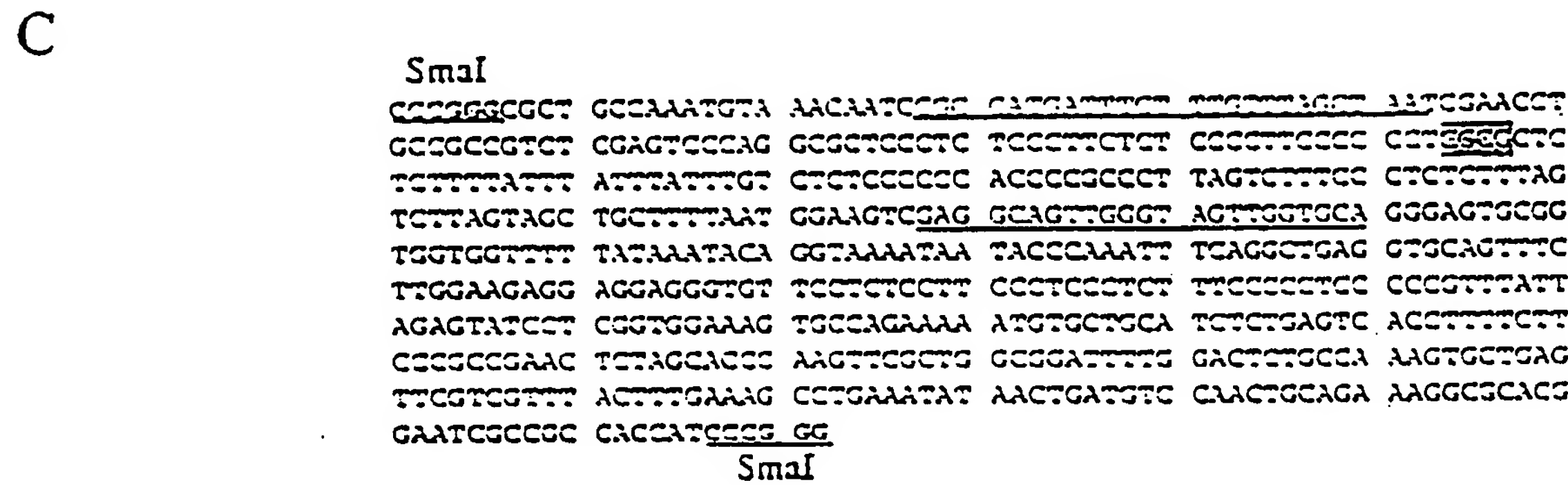


Figure 2



Probe : MINT2



MINT2 : 562 bp

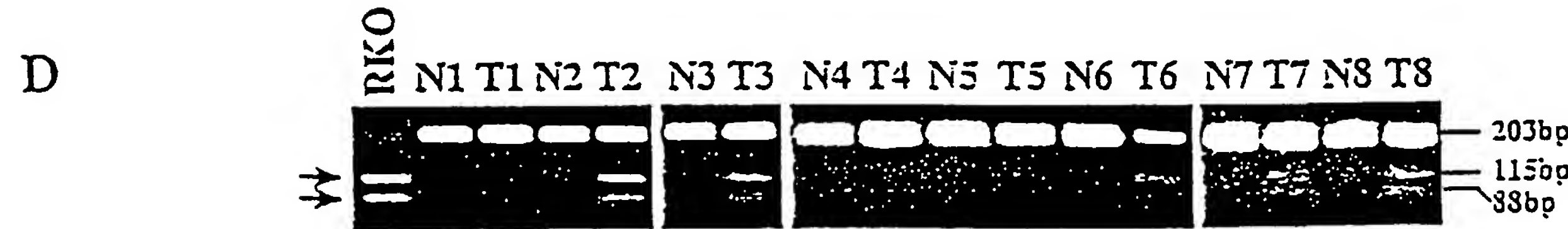
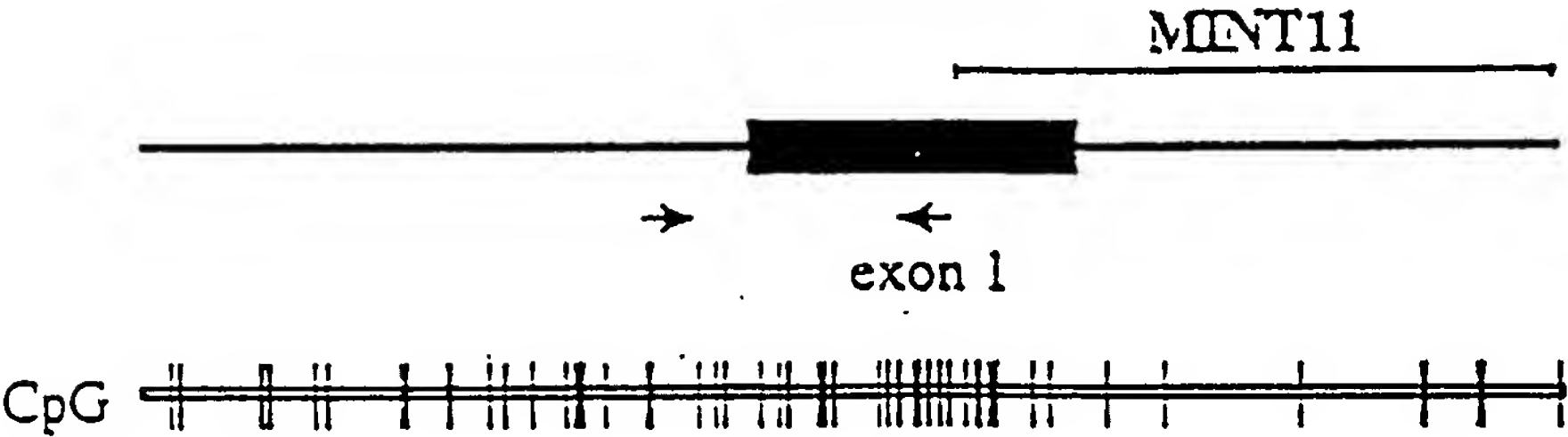
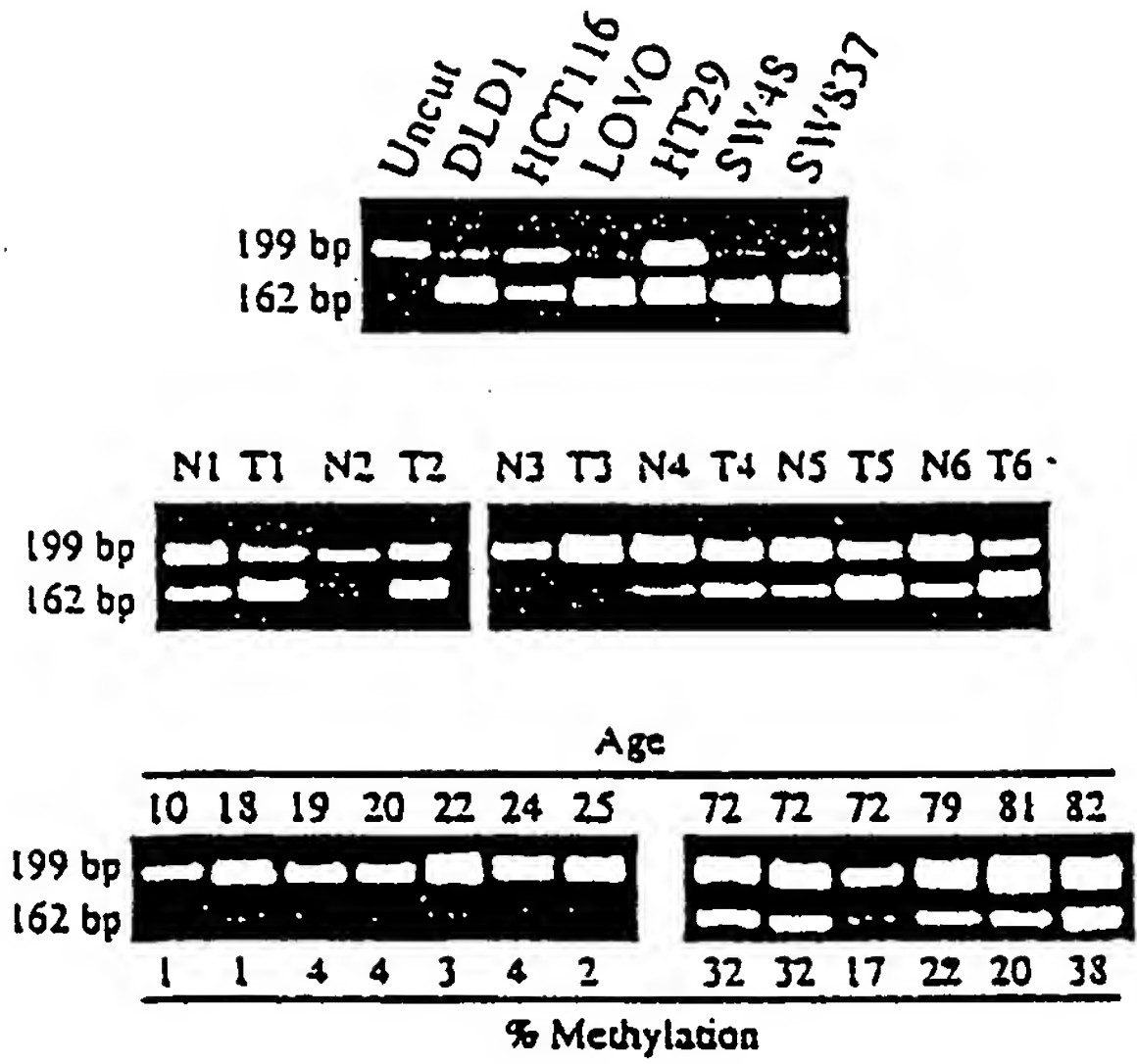


Figure 3

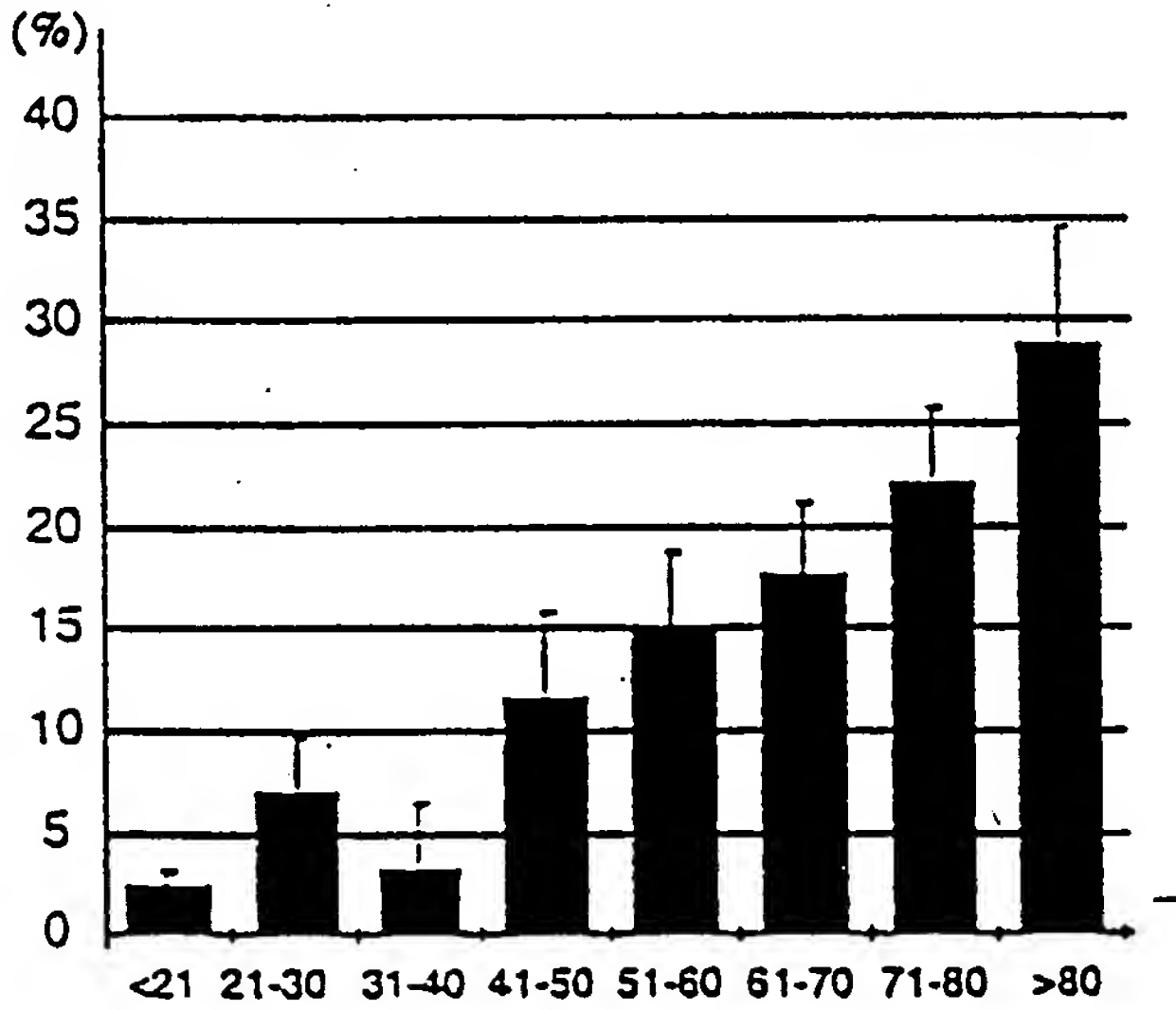
A



B



C



D

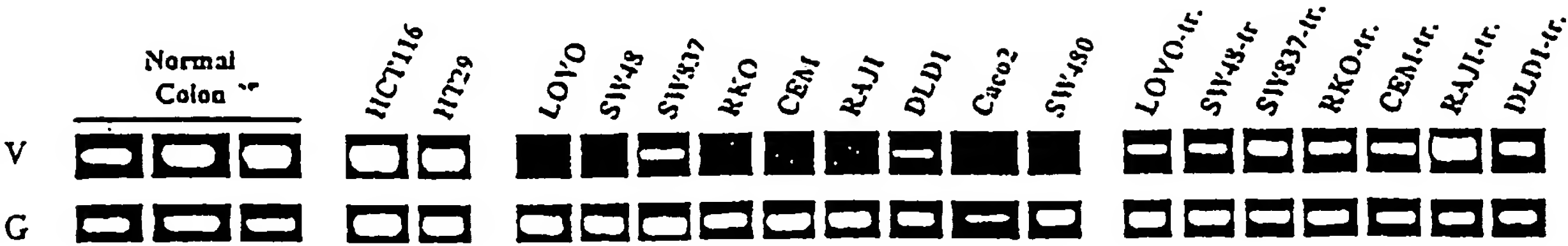


Figure 5

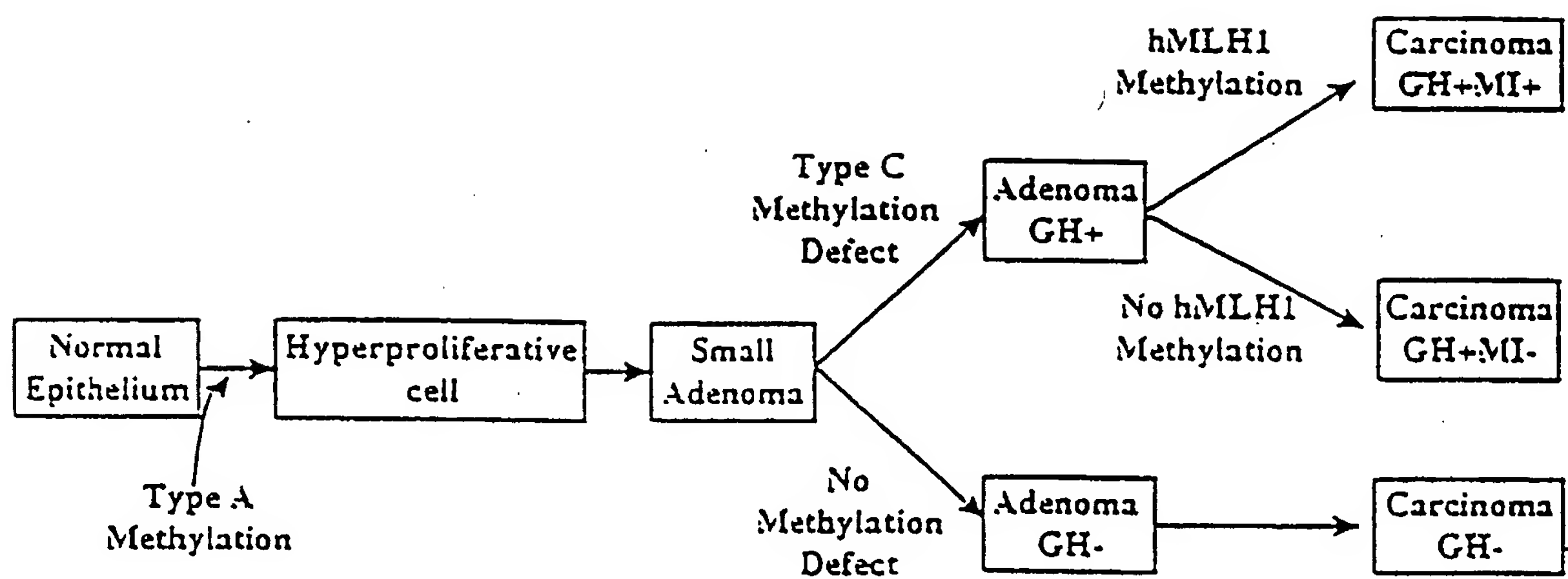


FIGURE 6A

MINT1 (SEQ ID NO:1)

CCCGGGCTGG	GTACCTGGAC	CTATACCTTC	ATAGCTGCCT	TAGGCTCAAC	TTTTCGGCGG
GGATCCCTCT	GCAGACGTGC	AGGTGGCGGG	AGAGCAGAGG	TAGCCGCAGT	AAGTGCTGAG
AGAGCCTGAA	AGAAACACCA	TGAATTTTCA	AACTCTCCCA	CATACATTCC	CGAAGCGCCT
GTCTGGCGTC	TAAGAGAGAG	CAAGAGAGGG	CTGGAGAGCA	GGGGAGCCCG	CGGGGCTGAG
GCTCTTTGTC	AGCGCCTGCA	CTTCCTACGT	TACAACGCCT	TCATTCAGCA	AAAACCTTTT
GGGCGCCTGC	TGTGCGCCAG	GCCAGGCGAA	GNAGACCGAG	GNTGTGAAGC	TCAGAGGGGA
GAGGGACCAA	TCGCAGTAAA	TAAGCTACCG	AGGTAATCTT	AGATGGNGAT	GAGGGCAGGA
AAAGNCATCA	GNCGACCTCT	GACCTTTCTC	TTAGGGGGTT	TTCCCCCTCC	GCCTGGGTTC
TAGAACTGGG	AAGANTTTTC	TCCAGAGCGT	CGCGGGGAGC	GCCCCGGG	

MINT2 (SEQ ID NO:2)

CCCGGGCGCT	GCCAAATGTA	AACAATCCGC	CATGATTTCT	TTGTTTAGCT	AATCGAACCT
GCCGCCGTCT	CGAGTCCCAG	GCGCTCCCTC	TCCCTTCTCT	CCCCTTCCCC	CCTCGCGCTC
TCTTTTATTT	ATTTATTTGT	CTCTCCCCCC	ACCCCGCCCT	TAGTCTTTCC	CTCTCTTTAG
TCTTAGTAGC	TGCTTTTAAT	GGAAGTCGAG	GCAGTTGGGT	AGTTGGTGCA	GGGAGTGCGG
TGGTGGTTTT	TATAAATACA	GGTAAAATAA	TACCCAAATT	TCAGGCTGAG	GTGCAGTTTC
TTGGAAGAGG	AGGAGGGTGT	TCCTCTCCTT	CCCTCCCTCT	TTCCCCCTCC	CCCGTTTATT
AGAGTATCCT	CGGTGGAAAG	TGCCAGAAAA	ATGTGCTGCA	TCTCTGAGTC	ACCTTTTCTT
CCCGCCGAAC	TCTAGCACCC	AAGTTCGCTG	GCGGATTTTG	GACTCTGCCA	AAGTGCTGAG
TTGTCGTTT	ACTTTGAAAG	CCTGAAATAT	AACTGATGTC	CAACTGCAGA	AAGGCGCACG
GAATCGCCGC	CACCATCCCC	GG			

MINT3 (SEQ ID NO:3)

CCCGGGTTTC	CAGCTTCTCC	CTTCCACCTT	TGTCTCCCCT	CCCTCCTACA	AACTTTCAGC
CTTCAGTCTG	TTGGGGGNTA	AGATCTGGGA	AATAAGCGTG	TGTGTCCGAG	TGCCTTAGGG
TCTGCTGTGA	GCCTGAATGC	GAGTCCGGTT	GGTTGTGCAG	TTATGAACCT	GTGTGTACAT
CTGTGTGCCT	AAGACGCTGG	GCAACTGTAC	CCACATGACC	GATGTGTGTG	AACGACTGTG
TGCCTGTGTG	TCTGGATCTG	CGCGTGGGTG	TAGTTCGTGT	GGCTGTGTAA	ACCGCATGCA
TAGGTATACA	TGTACCTATG	TCTCAGGCTG	TGTGCGTCCT	ACTGCGATGG	TACGAGAGTG
TGGGTGTGAT	GGTGTATGTG	ATCCTGTGTC	TGCGTGTCCG	TACATTTGAG	TGGGTGTTGT
GCGTGTGACT	GTGTAAACTG	CGAACATGTA	CGTGTGCCCG	CCCGTAGGTA	TTACCGTGTA
CGTGTGTCTC	CGTGTGCCTG	TGAGGGGTGG	GGTCTGCGCG	GGGATTCCCC	ACCCCCCCAC
ACTCACACCC	TCCAAGCCCC	GGG			

MINT4 (SEQ ID NO:4)

CCCGGGCCTC	TGGCCCTCTG	CGTCTGCTAG	NCTCTTTCCC	CCAAGACTCC	CCGAGGTGGG
GAGAGNACTG	GTGNTCCCTG	GAGAAATCAA	GGTGTCCAAC	ATTCTCTCCG	AGGCGAGGCT
GCTTGAGCGC	CAGCAACAGG	NCCTGCTGAA	CTTTCTTCCC	CGGCTCCTAC	GCTCCGGTTG
CTCTCCATCC	TCATTTCTGG	GGTCAAATGG	NAAAGAGGGA	ATACTCCTCG	ACCCCTCTCC
CCCTTGACTA	TCCAAAGCAG	CCCGAAGTTG	GCGAGGAGAC	TCTGCCGGGT	GTNCGGGCAA
ATGNCCCGCC	GGGTGGCTCC	AGAAATGGNC	TGTGANCTGC	ACTCGCCTCG	GAGAAATTCC
AACTCTTGGT	TGAAGACTCT	GACTCAGAGG	AGCCCTCTGA	GGATGCGCCC	CTGGAGAAAG
NGCACGGGAG	GGAAAGTGGA	GAGAACTCGN	CCTCCCCAGG	GGCTAGNCAG	CTACTCCCCG

G

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FIGURE 6B

MINT5 (SEQ ID NO:5)

CCCGGGTAAG	TGAGCCCTGC	TGCACTCCCG	CACCCCTCTT	CCCCATGCCC
ACCCTCCGGG	GATGCAACAC	CCTGTTCCCA	TGGAACACGG	GGGTTGGCAG
TCACACTGTC	CCCACCCAGC	TTCAGGCTTG	GTCTCCTCTA	GGTTTGCCTT
CTGAGGAAGC	AGTCCCAGGG	CATTTACTGA	CCAANCAGAA	AACAGGGGTT
GGGAAAAGTG	AGTAGGTGGG	TCTGCAACCG	TTACAATCAC	ATCACTTTAT
TCTTAATTTCG	AGTATATAAG	GATTGGCATC	ATANTGGGAT	GANGAAGGTT
ACTGTCCTTG	TCATTTGGGC	AAAAGACCCC	TACCCATATC	TCAATGACCA
ATTCCTCANA	AGTGTCCTCT	TGGAANAANC	TGANTTTTCC	CCTCCGTAAN
TCCTTAACAC	TCTCCGGCTT	CCCCTCAATC	CCAGGCCTTC	CCCCTATTGA
CCTCAAGACC	TCCTTTAATC	CCAAAGAGNC	GTTGACTTCC	NCCAAATGCG
GGTGCTTTCC	TTTCCAATCA	AGAATATGTT	TAAAAACCCCT	CCCAGGGAGT
ATGGACTATG	TCCCCAATTT	TAAAAGATGG	AGGAACAAAG	GCCCATTGGT
ATGCTAAAAA	CCATGGGAAC	AGGATCCAGA	TTTCCCCCCA	TCAATTCGAN
CTGCCAGTCT	GTCCTCGGAG	ATCCTTTGAC	TTCTTGGAAT	ANCCTTTTGT
TGTTGTGGTT	TGGGGGTTGA	TCTTGAGAGAA	CTTTTTTGTG	TGTCTTTTAA
AAAATGTTTC	ATTNGTTAAC	TTTCCAAGTG	ATGCTCTGAT	TGGAGCAATC
TCAACACCAA	GAAGAGTAGG	GAAAGAAGCA	GCGGNGGTCC	TGGGTCCCCG

GG

MINT6 (SEQ ID NO:6)

CCCGGGCCCT	CAGAGGCCGC	ACCACCTATT	GTGTTCCAGG	CTCGCAGGAA	GCCAGACCTT
GCGAGAGGTG	TGTGGGGGCG	GAGAGTGGCA	CAGGTTTGAC	ACTGCAGGTC	GGAGGAGGAA
GACAGTGGCT	GCAAAGGCAA	AATCGGGTGT	TATTTTCCCA	AGAGTCCCTT	CAGCGTGAGT
GCCGGGGTCA	GCTCGAACTG	GAGCCTGTAA	TTTGTGAGTG	CGAGTGGGGA	GCAGCAGGAG
GATCCTTTTC	ATAGGTGAGG	TCCCAGGAAC	GAGCCTGGTC	NGTGCTTAGG	CAAAGGCCCT
TCCCACCTTG	CAGCCTTGTT	GTTTACCCAT	CCCCTGCTTC	TCCCAGACTT	GCATTAATTC
CGNNGTCGGT	TCCATCAACC	CCGGNATCCC	CTCCCCCGGG		

MINT7 (SEQ ID NO:7)

CCCGGGACCC	GCCCAGAACG	CTTTCGTGGG	GTTGGAGAGG	GCAGGACACA	GCCTCTCTGG
GCCCGGTAGT	ATGCGAAGAC	ACGCATAACG	CAAAAGGATT	CCCGTCCTGG	ACTTTGGGAA
TTAAGGCCCT	GAACACATTG	AGCAAAAAGT	AGATCTGTCT	GTACAGACGT	TTCTTTCCAC
GTCTTTTATA	GTAAGGACTT	TATTAAAAAG	CAGGCACTCG	AATCCTAGGT	GGGTAGATGG
GAGGCTGGGG	CTGGGGATGG	GGACGTCCTC	TGTTTTCTGG	TTGTGCACAT	TAAAAATAAC
TCTCTGCTGC	CTACTCCTAA	ACGCAGCCGG	CAAAAATGAG	ACGTCAACTA	AGCGCCGTTT
CAGTCCCAGA	GCCAGGTCGT	CCATGGGGGT	TTTCAAGCGT	TTTCTCGATG	ACTGATTTTT
CTAGAAGCGA	ATGGATTTTA	TTCTCCCAGG	TGTAAAGGCT	ACCTCCTGCT	TCACACCCGG

G

MINT8 (SEQ ID NO:8)

CCCGGGCTGT	GGCGGTGCAA	CTCCCGCCGC	CTGCGTTCTA	GACAGAAAAG	CCCCTTCTGA
CCATGCATTA	GTAGTACTAC	TCCATTATTC	CTGTTAGAAC	AAGTTAAAAG	TAAGGGTTGA
GCCCAAAGGC	CCCAGGAGGG	GGGTCATGTG	CGCCCCAGTC	ACTCAGGCTC	CCCTCGCTTC
TCCGGTTCGA	GTTATGCCAT	CAAGCTAATA	TATTGTGACT	GCTCTTCTCT	CCTGTGACAA
AAGGCTTGCA	GCTGCCTCCA	AATCAATAGA	TTGTCAAAGA	AATATTGAAA	ACAATCATGA
CACAAAAACT	TAATCCTGGN	TTGGAGGCTA	CATAATCAGA	AATTGTGCTA	CTTGTTCTTC
CAAGNCACCC	GATTTAATTT	ATCCCCAAAC	TTTTAGGCAA	ATTTTTATTT	CCCGGGACCT
TTTTCCCAGC	AGATCCTGCT	ACGTCTGTCT	GGTTTGTAAT	GTAATTTGTA	ATTNCTNCCC
TTCATGTGGT	CCGGTGCCTT	GAACCATCTT	TAATTAAGAG	CATAATTAAG	GGAAGATCTA
AAGAAAGACA	ATTACCAGAT	GGTCTTTTTT	TTAGAGGCGG	TAGTTGCGCA	GAGAGGGGCT
CGGGGTCTGT	CCCCGGG				

FIGURE 6C

MINT9 (SEQ ID NO:9)

CCCGGGTTCAT TGTCCATCTC CGACCAGGGG AGTAGCCACC CCCACTAGCC AGCCGTCTTT
ATCTCCTAGA AGGGGAGGTT ACCTCTTCAA ATGAGGAGGC CCCCCAGTCC TGTTCCCTCCA
CCAGCCCCAC TACGGAATGG GAGCGCATTT TAGGGTGGTT ACTCTGAAAC AAGGAGGGCC
TAGGAATCTA AGAGTGTGAA GAGTAGAGAG GAAGTACCTC TACCCACCAG CCCACCCAGT
CCCTCTCTCA GCAGTGGATG GGGATGGGGT GGGGGTAGGG ACGAGAAGGC AGCTGGTGGA
GAAACAGCTT CAAGACTCTT TGGGTTCCTC CTGCTCTCCA GGGGAGCTTA CCTGGGGCTA
ACTTTAGACA CACAGGTTTG GAGGGAGGAA AGAAGGAAGA ATTCTTTTAC AACGAATCAA
TTAAGAGCAC TTCCTCTTTT CTTAACTTGG GGGGAGGGCC AGGAAAACCTT CTTGAGTCAA
GAAATCTTGG GAGGNAGACA TCAGATGTNG GCAAGAGGCA GACAGATTTT GGGAAGGCAG
GCTTTGGGTC AANAAAGATC AAGCCTGTGT TGTCCCCAGG CTCTTCCCTG TTCCCCCATC
CCGGG

MINT10 (SEQ ID NO:10)

CCCGGGGGCT GCAGAATCAG GAGTCTTCNC CTAGGTTTGG CCTTGGGCTC CATCCTACNC
CCTGAATGTG ACNGCTGCGT CTTCTGTCCTA CACCTACTTT TGAATGCCAA GAGGGGGCTC
CGCTGGCCAG GACNGAATAT TTTTATGGTA AAAAATGACC GGCAGTTGCA TCAGCTCCAG
GAGGGTGGGA GCCGTCACCC GAGGTCGCAC AGGCAGACTG ATGAAAATTC TGCTTATAAA
GTCACTGCTC CCCCATTAAT TAGGGGGGAG GGGGCGCTCC GGAGCCACCA CGCACCTCGC
CCACGGNCAA AAAGCTTGTC AACATTTTCC ACGAAGGATT GAAAATGTAA ATTAACCTTC
AGATTATTCA ATGTCACCAA GGTATGGAAA AAGGTCGCCA TACTGGGTGT CATTTATCTC
GTTGTGGATT TAAAGAGCTT TTTCTATTAA ATTTCTTAAA ATTAATGTTT TATGTTGCTC
AGAGTAATTT AAACAATTAT GGGCTTAAAG AATTGATCAT TACAGCCCCT GGGATTTAGC
GCTGCAGGCT GATNNCCCTG AAAAACCTCT GATTTATCAG GGNTCGTATT NGGCCGGGCA
AGCCCCGGG

MINT11 (SEQ ID NO:11)

CCCGGGAGTG GCTAACCAGG AANANNAGGC ACTGNCCACA CACCANGGGC TGGGAAATCA
AGTGGCCTGC ACCAAGGCGG CTTCTGGGGG CTTGTCTGTG GCAAGTCTTG GTAGTCCCCA
TTCAAACCTTT TGCCTCGAGC GTGTTAAGAA CAACAACAAA AAAAAAATCA AAGTGCCAAA
GGTCCCTCTC TTCTCTCCAG CTCAAGAACC CACCACTTTT CTATGATTTC TTTACAATTT
ATTCCCTCCC TTCCCCCAAT TCCGTTAGTC ACTTTACCCC CACCCACCCC TGGGTTTCTT
TTGTCTGAAT CTTTTTCAAC ACCAAGGTCC CTCTGTATGC CTCTCCCCAA AAGCCCTTAT
GAAAAGTTAC CTGCATTTTT TAAGTGCCTA CATTTCTTAA CTTGCGCTAA CAGCTCTTTG
CCTTAATTAA AGCCTTCTAC CAATTGCTTC TTTTTTCTAA GCTCGCGGGT TTTTTTCAAT
AAGTTTTTTG TTTTTGTTTT TTAAGGGGGG AACAAAAGAA ACGTGATTAC CTTGGAAGGC
GGCTTATTGC AGTTTGGGGG GAAAATTCAC TGCAGCGCTG CGCGACTGGG TTCGGCGTTG
CCCAGGCGGG TCACATAGGA AGCGTGGTGG CCCGGG

MINT12 (SEQ ID NO:12)

CCCGGGTCCC AGCCCTGAGG ACCAGGTTTC AGGGCTCAGA AGACTCCAGC
GAGGTTCCCT CGCAGATTGT GTCTGCGGTC GTTGGGGGAG GGGCCCCGCA
GCCTTCAGCA GATTATCCAA AGGTCAGTGA CCCAGATATG GTTTTGGNCA
CGGGCCATGT TTTACTTCCT GTGCCCCAAG CAGAATTTAG CTGAATAATT
CGGACCCCAA ACCAAACAAA ACGCTCTTAT TTCCGTTTGG GGATTCTTCG
GAGTTGGGAT TTTTCTGTCT CAAATTAGAA TAATNTGCAT TATTAACCAA
CTTAACATTT ATTTGTTGGT TGGCTGCCTG ACCTTTCTGA GCCTCAGTTT
NTTCGTCTGT AAATTGGGAG CTTACCCAGG TCGGAGGACT GTTGGAATTG
GAAATATTCG AATAAGGAAG TGTTTTTGCA AGTGCTTTGT AAGCAGCAAA
GCGCTTCTTC AGGGGTCAAT TTTTTTTAGC TCTGCAGTCA CCACCCAAAT
TCGGAAGATC GTTGTGCCTT TCTTGGATGA GAATGCCCGG CTCCAGCCCCG
GG

FIGURE 6D

MINT13 (SEQ ID NO:13)

CCCGGGTGTG TGTGTTCCC CTCCATGTAC CACGTGTTTG TCCTGATGGT CTCCTACCCC
CTGTCCCCCT GAGAGGCCCT GGTGTGTGTT GTTCCCCTCC ATGTATCCAC GTGTTTGTCC
TGATGGTCTC CTACCCCCCG TCCCCCTGAG AGGCCCTGGT GTGTGTTGTT CCCCTCCATG
TACCCACGTG TTTGTCCTGA TGCTCTCCTA CCCCCTGTCC CCCTGAGAGG CCCTGGTGTG
TGTGTTCCC CTCCATGTAC CCACGTGTTT GTCCTGATGC TCTCCTACCC CCTGTCCCCC
TGANANGCCC GGG

MINT14 (SEQ ID NO:14)

CCCGGGAGTG GCCCTGCCTG GCCATTTGCT CAAGCAGCAT GCAAGCCGGA TCTACAGCCG
GTGCACCTTG TTCCTTGTTT TTCCGGGCAC CGGAGGCCCA CGTGAAACCT CTCAGAGGAG
AGCGAAGAAG GCCACCTTTC ATCTCAATGA GCCAACAGCC TCACATCCTT AAGTCCTGCC
TAATCTTACG AGTCATGAGT CATCGCTTCT TCCCCGCCAA GTCATTGAGG ATTCAGTGAC
TCCAGCTGCC CTCAGGATCT GACGGAATCT TGAAGGCAGA GTTCCGAGAC TGCAGTCCAG
GATAGAGAAG CCCCCGGCTC CATCAGGGGC TCCTCGGCTT CAAGGCAGGA CCCACCCCAA
AGCTNTCAAA GCGGCAGAGG CCTGTTTTCA GGTCTATTTT TAAAGATNTC TAGGGGAAGT
GGTTTCTGAA TGCTCTGTAG GATGAGGCTG TGAACAGTGA TTGTTTCATT TGCTCGGGGC
TGGCAAAAAA GGGATGTACA ACCCGTTCTG ACCACACCAG TGAGTTAAAA AGCACTGCAG
ACTATGAATA CCTTTTGCCA GTTGGAAGTG TTATGGGGAC CGATGGCTTC CCCTACAACCT
GGGGAGGCTG CCAGCCCCGGG

MINT15 (SEQ ID NO:15)

CCCGGGACCT CTGCGGTTAC CTGGGCCTTC CCTGCCAGCC CCCACCCCCT GCCCCACCA
GAAAGCGTTT ACAGGACAGG TGAGGCCCGC AGGAGGAAAA GCACTCCCCT GGCGCAACAT
GACTCCAGCG CATCTGCGTC TAAGCCACAC CGTGCTCCTG GTAGATTAAA AATTAATTCT
AAAAAATAAA TCTCTCCTAT CCCAAATGCA CTGTTTTCTG CCTTGCTTGA CAATTGATTT
GTTTTTAAAG GAAAGTTATG GGTAGATCCT CTTTTTTCTT TCCCATTCCT TNNTTCTTCT
TTTATACTGG AGGGAGGGAA ACGGAGGCGA GGACACACAC GCGCAGGCAG GGGNTGAAAA
GGCCGAGGTG GGTTTTCTG TTTAATATCA AAGGAGGGCG AATAATGGGT TTCCTCGGTC
CGGCTAGGCC GGCCTTTGAC TCAATTGGAA ATGCAAAGGC AGCTTTTGCC TATTNTCTGG
CTGCTGGCTG AGACCCTAAA TTTCCGTAGG AAATCGTCGG ACACGCACTT AATCGGNCTT
TGCAANCTTT CCCTCGAAGT TGCACGCGGG TCTGGGCGGA GGAGGCGAGG TAACCCTGGA
TTCGAACCAG CGCCTTTCTC TCCTTCAGGC CTCCGCCCGG G

MINT16 (SEQ ID NO:16)

CCCGGGACAA GGCGGGTCAC CTCTGGGGCC TCACCGCAGT TCCACTTCCT TTCTCGGGTA
TTTGGAACCC GTCACCCCGC CATTTGCGTG TGGGAAGAGC GCGCGGGCCC TGCCGGACTT
TAGTGCTTTA GGGGTTAATT TCGGGCTGAC AGGGACGGAG CCTAAGGCAG TGAGCGCCCC
AGTACCCTCA AACCTTATTG CTGGCCCCCTG CTGTCTGAGC TTACAAGCAT TACCGCCGCT
ATTTCCGTGC GGGCTGACAC GGGAGATGAA AGTGGTGAAG ACACCCAGGG TGCGGGGGTG
GAGGTGGGGA GAGGAGCCAG ATGGGATTGA TCCCCAGAGC CAGATGGGAT TTAAAGGTGA
GGGAGGAGGG CATCCTGATG GCGTGTGGTC AGTTGATGCC AGATTGGATG GCTGAGACAC
CTCTGCAGCT TACAGGAAAG ACAGAGGGAA AGGGTTCTAT GAATTCTAGC TGTTCTACT
CAAAGCAAAT AATTAATCAA GTGGGGGGGG GGCCTCTAGC TGTAACCCCA TACCTCTAGG
AAACCTTTTG TCATGTGGAG CCACAGTGCT CACTTGACAG ATTCCCCACT GAGAAGTGGG
CTAAGAGGTT GGCCTGCATT GCTGGGTGCC TCCAGGTGGG GAGTCCTGTA CCTGGGAGCC
CGGG

FIGURE 6E

MINT17 (SEQ ID NO:17)

CCCGGGCGCC GGGGGTCCCA TCCACTTCTC CCTACCTTCT CTCTCTCCTG TTGGAGAGGC
CAGGGGCTCC CGCGACTGAA AGGGGCCAGG CTGAGGCTGC CCAATCCCAG GGACAGGAAG
AACATTTTGG ATGGATCGCG GGGTGCAGAA AAAGGAAGTT GAGCGACAGA CGCNAAGCCC
AAAGCGTGGA ATTTGGGAAG AGGCAGAAAA AAAGTGGGCG AAGAGGAAGA GAAAAAACNA
GCNACGAGAT GGGGAGGGAC AAGAAGTTTG GGGAGGATAG AGAAAAAGAG AAAGTGCCTG
GAAAAACAAGT TGCAAGACAA TTGAAAAGTT GAATGAGAAG GAAAGANAGG AAGGTNGTNA
AATCAAATTA GAACTGTTGG AAAAGANGGC TGGGACACGG CTTTCTCTGG TTCTGTCCTC
CCAAGAAATT GGAAACCTCT CCCCTTCCCG GCACCAANCT TNCGGGATGT TCCGGTGCCC
CTCCCCCGGG

MINT18 (SEQ ID NO:18)

CCCGGGAGTG CTCCTGCCGG CAGCATGTCC ACTTGCTAGG GGCAGAGGGG CAGTGGGAGT
GTCCCCCATG TGCCAGCCTG TCCCCACACT TGGGTTAACC TTCAGTCACC AGGAGGAATA
AGGTGTGACA CCTCTGAGAC TGTGGGCACA TGTGCTGCTG CCTGACAGTC ACAGGCTGTA
CTGTGCCAGT GACAGAGGCC ATGATGCCCA CATAACCCAA ACCCGAACCC GAACCCTAAC
CCCTAACCCC TAACCCTAAC CCTACCCTAA CCCTAACCCA GCCCTAACTC TAGCCCTAGC
CCTAGCCCTA GCCCTAAGCC CTAAGCCCTT AAGCCTAACC CCAAACCCCC AACCCCAACC
CCNAACCCTA ACCCTTAACC CTTCCCTCAA GCCTCTCNAA CCCTGCTTGG GTTTACAAGG
TTATTNAACC CCGGG

MINT19 (SEQ ID NO:19)

CCCGGGATTG GTCTTTTGGC TGGGATGTAA AGGAGGAGGA ACTAGCTGGG GAAAGGCTGG
GTAAGGGGGA AAACCCAGGG AATTTAACCC CCTCTTCTGT AAAATGAGAA CCCATTTCATT
CACTTAGCAA ATCTTTATGG AGCTCTGTAG GGGCCCTGGG GGAAGGCGAC CAGAGTGTCT
GAAAGCAAAG AGCAGGAGAG TGTGGA CTCA GCTGAGAGGA GAGCAGAGGC TGAGTGTCTAG
GGTGGGGAGT GGGAGGGGGT TTCCAGGTGC CAACAAGGAC ACAAGCCAAG GTGTTGCAGT
AGGGCATT TG GGGAACGTAT GAGAAGCGCT CCTGCCAGCT TCCCTGGCGG TGTCTATTC
CCTGCATCCA TTTTGGACCA TGAGCCCTT CTCTTACCCT CTGGCCAGGA CCGAATGCCA
TAGACTTCCC CAGATTGCCC GGG

MINT20 (SEQ ID NO:20)

CCCGGGCCCC AGGGCGGCCC GAACCCCAGC CAAGCCGGCC AGCAGCAGGG CCAACAGAAG
CAGAAGCGCC ACCGGACGCG CTTACCCCC GCACAGCTCA ACGAGTTGGA GAGGAGCTTC
GCCAAGACTC ACTACCCCGA CATCTTTATG CGTGAGGAGC TGGCACTGCG TATCGGGCTG
ACCGAGTCCC GAGTGCAGGT ACGAGGGGCT TGGGATCTGG GACAGAAGGC AAGGACAGGG
CGGGAGGATT TGGGCAAAGG GAGCAGGGTC TTCCCTTCCC CTGTCGAGAT CCTGGGCTGC
TTTCAGGCTG CCTGTGCGTT CCTGTATCGA GTTATCTCCA TCTCTACCCG GAAACTGGTC
CCCATCGCCA TCCCCCAATG GACACGCAAG GCCCGTCTCC GGCCAGTATA GCGACATCCC
GGAAGAAGCT CCTCAAAATC GAAGCCCGGC GTTGTCGGGC TACAGGGCTC GCCTCCTCCG
CCTGAGAAGG CAACCTCAGC GCCCCCGGG

MINT21 (SEQ ID NO:21)

CCCGGGAACT ACCTAACGCT AGTTCAGTCC CAAAATGCTG CCCAACGACA GAATGCTCGC
CTCCTTGCTT CCTCTAACAC TCTGGCACAC CCACTTGGTG TCGGGCCTCT ATGGGCTCGC
AGTGAAGCCC TGAGCCTGGG CTGCCCCCTC CCATGTGCC CCTGCCAGCC GGCCCTCCCT
CCCTTTGGGT GCCCCATCCC TCCAGTCAAC TCCTAGCCGA CCCTTAAGAG TCAGGTATTT
GTAGCCTTCC CTGACATCCC TCCCAGGCTG TCCCAGTACC AGCAGGACGA GCCTGCCCCCT
CCTCCACCCT CCTTACAGCT ATACCTAGCC TTGGCCATAA TCACTAATGG ACCAGGAAAC
ACCCTGGCGC GCAGAGCCAC CGCAAAGTGG CCCGCTCAGG CCCGCCCCGGG

FIGURE 6F

MINT22 (SEQ ID NO:22)

CCCGGGGATC	TCAGAAAGGG	AAGGATGTGG	GGAGAGTGAA	GGTGGAGGCA	GTCACACCTA
TCCTGGTGAC	CTTGGCGTGC	CCCCCTGGAG	TGACGAGCCA	GGGGCTTATA	TAGGTGCGAC
TAGGATGTTG	CCTCGTTTTT	CACTCGGGGC	TGCGAAGAGG	GCATTGCTCT	TTCTGATGGC
TGGAAGACAG	GGGCAAGGAG	AGAGAGAACC	GGCCCCGAGA	CGGGCTGGAG	GGTGGGGACA
CTGGGGAGTT	TGGAGCTGGG	GGTTCGGAGT	GGGAGGTTTG	GGTCTTCTGA	GACGCTCCAG
ACTCTCCGGA	GGCGGCAGAG	GTCGAGGCAG	GAGGCGAATG	TGACGCTTAG	GGTCGCTACG
GTTGATGTTG	GGCGCCTTTG	GAAGCTGGTC	ATTAATTCTT	GTCATCGGGA	GGTTTCGCGG
ANGGCGACAG	CGCCCCGGG				

MINT23 (SEQ ID NO:23)

CCCGGGCGCC	CGGCCCTGGC	TCGCGGAATG	GGCGGCCAGA	TCTCAGGCC	TGCGTGCCCG
AGCTCAGTCC	CAGTTCCAAC	CGGGGGTGCC	CATGGACTCT	CGGAGGGCAC	TCCTGGGGGG
ACAGCTAAGA	CACCAGGCTG	CAGGATCACT	CATTGCACGC	TGCATAATCG	CCGCCACAAA
CTCTCCCGTG	CGCAAGAACA	AACGCGCGTG	GGACAGAAAA	AGTTCCTAGG	TCTCCGCAGG
AGTGAATGCA	AAATCCAGGG	GACTCAGGGT	CATGTTGGGA	GCCCCCTTCTC	CCCCCGAGAG
TCAGGGAGCT	GTTGAGGTGG	GATCGGTGAG	GGTCGCGCCA	CGCGGGTCCC	TTCCCTACCA
GGCTCGGATA	CCATGCAGCG	TGGACACTCC	CGAGTTGCTC	TGCGGAATCC	CGGG

MINT24 (SEQ ID NO:24)

CCCGGGGACG	GGGAGGGAGG	AGGGCTGCCG	GGATGTGAAC	CGGGGAAGGC	AGCTGGGGCT
GGAGAGCAGC	GCGGAAAGGG	GGCCCAGGGA	GCTGGAAAGC	GAGCCAAGAG	GAGGGCAAGG
AAGGTGGCGG	GCTACGGGGA	GGGGAAAGAA	AAAGGGTGTC	TTGGCGGTGG	CCTTGGTAAG
AGAAAGGGGC	AAGGGGTATA	ATTGACAAGG	CACTGAAAGT	ATTGAAGTCA	GAGCCTTGGG
AAGGATCTAC	CGAACTCTCG	GCGGTCCACG	CGGGGACAGA	CCTCAGCCCC	TGAGCCTTGA
GCTCCACGCG	GGGACAGACC	TCAGCCCGTG	AGCCTTGAGC	TCCACGCGGG	GACAGACCTC
AGCCCGTGAG	CCTTGAGCTC	CACGCGGGGA	CAGACCTCAG	CCCGTGAGCC	TTGANCCCAG
AAGGAGTGGC	AACCTCANGA	CGTTTGCCAA	GTGGCCTGGA	ATGTTANGGA	AACCCCAGCC
CCGCCAGGAA	CANANCTGGC	ACTAATTCCC	NGCTCGGNCC	GGG	

MINT25 (SEQ ID NO:25)

CCCGGGGTGG	GAGCTGGCTC	GTGGGGGCGT	GCGCTGCGCG	AAAGCGAAAG	CCGCCCCGCA
GAGCAACTTT	GCGGCGGAAG	GCGCCGACGA	GGAGCTGTGC	CGTGCCGCTC	TTGGGGATGG
TGAGCTGGCC	GCCCCGCCGG	GTGGGCAGCG	CGTCCGGGCG	CGGTGCTTCG	CTAGCTATAA
ATAGGTGCTG	TGCGGGGACA	GGAAGATGGT	TCCGGCCCTT	TACAAGCACC	GGCCCCGTTAT
GTGCGCTGGG	CTAGGACCTT	GCCCCGCAGC	GGAGTGGGAG	GAGTGAGGTT	AGGGGTAACG
GTTGCATGGG	ATGGGGGGTG	GGCACATAGA	GCCTACAGCA	GAGTTGGCGG	CGGGGCTCTC
CCATGCACTT	GGTTGTTTGT	CGTTTCTGCT	TTTCCCCGGG		

MINT26 (SEQ ID NO:26)

CCCGGGCTCC	GGCATAGCTC	TAGATTAACG	AGCTGGGCGA	CGGGGGCGGG	GGCAGCATGC
CCAGCGTCGG	TGCACGGCCG	GGGTTCTTAG	ACATCACAAA	CTGTGGAGCG	ATACATTGGA
AGCGAAATCC	AAGAACGACA	CCGGCCGGCG	TGTTTCTGAT	GTAGTCGTGA	TTAGTGTTGG
AGATGGCCAA	GGGCGGCTTG	CGGAGCCCAG	GGAACGCAGC	GAGCCAGGCC	CGCGCCCCCC
TGCAAAGCTC	TGCCTTGAAC	CACGTAATTC	AGGCACCCAG	GGTGTCCCTC	CCTAGGTCCT
GGCCACATTT	CCCGAAGGTT	ATTAAATGGA	GAATTCAGCA	GTGGAGTTAG	AGACGGACGA
ATGTACAGGA	AGATAAGAGG	GAAACTCTTC	CTCATTTGCT	TTAGGGGGTT	GTGCTGGGAA
AATGCGGGGA	GGTTAAACAA	AGCTTCTCCT	ANGACTCCAC	GATTCATTTT	TAACAACTTC
TTAAAAGACT	CCCGTCTCNG	GAGCAGACGC	NCCCTCCCCG	CTCTCTAAGC	CCCGCTGCAT
GAAANGATGC	CCTCGGCCCC	TTGCCAGAGG	GCCGGGTCCG	GGATTCCCCG	G

FIGURE 6G

MINT27 (SEQ ID NO:27)

CCCGGGATCC GGAAGGCTC CCGGAGCCG GGTTCGGAAC TCGGCTGGA GGGGCCTCGG
CTTGAGGAGG ATCTGGGAGG GCGGGGGCTC AGTCCTGGCC ACCAGGTGTG AGGGGTCGGG
TGGGAGCCC TGTGTCAGAC GCGGCGGTGA AGGCTGTAGC CCTGCTCTCC GGGATGGGGG
TGGTACTCTC ACCGCACCCT GCCCAAGCC GCAGGGAGCC CCTGGCGCCC CTGGCGCCCC
GG

MINT28 (SEQ ID NO:28)

CCCGGGCCCC TGGCGGGGAC ACCGGGGGGG CGCCGGGGGC CTCCCACTTA TTCTACACCT
CTCATGTCTC TTCACCGTGC CAGACTAGAG TCAAGCTCAA CAGGGTCTTC TTTCCCCGCT
GATTCCGCCA AGCCCGTTCC CTTGGCTGTG GTTTCGCTGG ATAGTAGGTA GGGACAGTGG
GAATCTCGTT CATCCATTCA TGC GCGTCAC TAATTAGATG ACGAGGCATT TGGCTACCTT
AAGAGAGTCA TAGTTACTCC CGCCGTTTAC CCGCGCTTCA TTGAATTTCT TCACTTTGAC
ATTCAGAGCA CTGGGCAGAA ATCACATCGC GTCAACACCC GCCGCGGGCC TTCGCGATGC
TTTGTTTTAA TTAAACAGTC GGATTCCCCT GGTCCGCACC AGTTCTAAGT CGGCTGCTAG
GCGCCGGCCG ANGCGAAGCG CCGCGCGGAA CCGCGGGGCC GGG

MINT29 (SEQ ID NO:29)

CCCGGGAGCT GACCGTGGGG AGGCCGGTTC CGCTGGTTTC AACCAGCCCA CTCTCCCCTC
TTGGGATGCC CAACCCCGCG TACTCACCAT TTCCTGGTTT CCAGGATGTC CTGGCATCTT
AGCTATGCAT TTCCAGTACC TCCAGACCTC AGGGCAACAA AGGATGTGAC AAAGTCACCT
AGTGCTCCTG AGGGGCAACA CGCGGGACAG TAGAGATGGA TCTCAGGCTC CGGCTGAGC
CAGAGACAAA GGCCGCGCCA AACGCTGGAA GCCACGCCCT CCTCCCCAAC TCGGTGCCTG
ATAGGACGGT TCCTACTCTG ACAGATTGAA TAAGGCTCCA GGACCCTCC CCACACCCAC
CGTCCCCAGC ATTAGTGCGC TTTATGGACA GGGAAACGGG ATCCTGTANG CGGGGTCA
CGCCCCGGG

MINT30 (SEQ ID NO:30)

CCCGGGCACC TGGGCTGGGG GGGCACTCAC ATGGCTACCG GAGGCCCCCA CGTGCGGCGC
CCCGCGGAGA CAGGGGTTCG CGTTCAGAGC TGGTGGCGGA TGGACCAGGT GGCCGCGGGG
ACCAGCTGGG TCCAGATGTG CTGGGCCTGC TGGAAAGGGGA CAGGTGCTAC CTGGACGTGT
AATGGCCCTT GGTCTCTTTT GGCCGAACCT GCCGCTCCGA TCCCCCTCCA TCCCTTCATC
CCTCCATCCC TCCATCCCTC CATCCCTCCG GCCCTTCTCC CCTTCTTCCT CCGCTGCCTG
TGTTGCAGAG AGGGGCTGTC AGAGACTGTT GATGTGGGAA AAAATGAAAT GGGGGAGGGG
TTGTGATTGG CAAAGGCCAG TTGTGCCGGG AGCGGTGGGT AGAGGGGGTG CCCTGAGAGT
GGGAAGCCCT AAACCTGGAG GGCAGCGCTG ATGGGGAGAG GGTTCCTGGC ACCCCACCT
GCCTTGGAAG TGGGAAATGA CATAGCGGGA GGGGGGCTGC AGTTCCAGCC CCCGGG

MINT31 (SEQ ID NO:31)

CCCGGGGCCT CTATCCTGGC GGGAAGGGCA GGCCGACCCG GCAGACTGCG GCCTCTCGGG
AGGGAAGAAG GTGTCAGACG CGCGGAGCAA CCATAAATAG CCCCCCTTTC CCAGAAGACG
GCACGGGGTT CAAGACTCAG GCGCCGCATA CTCAGAATGA GAGCAGAGAC TCCCGCCAGG
AAAAAAGGGC ACTTAGGGGA TCTGCTCATT AACATGAAAT GCAAATGAGC CCGCCCGGCC
TCATTTACAC AACTCTGTGC ATGGATTTCG CGAAAGGGCA ACCAGGGAGA CGACGGCGCA
GCAGCCACTC TGCCACTTCC CCCATCCCCT CCCCCCATC GGCCGGGGCG GGAAGTGA
CGACCCCAAC CCTCTGCGGC GGCGGGAGGT GCGCGGGGGC TCGGTGGGTG GTGCAGCCTT
AGGGGAGTGA ACAACGCCCA GGGGTGATGG CCTCAGCAA GTGAGGGGTG GTGATGGAGG
TCATCCGACC CATCCCGCCG CCTCTCCGCA GTGGCGCAAG CGCCCCAAA TCTCCGGAGA
NGGAAGTGA TGACCCACTA GGTTCGCGC GTGTCTACCTC TCGCAGATGT TGGGGAAGTG
CTTCCCGGCG TCTAATCCTC GCTGTTCCCC CCTCCACCGG CGCCAGCAC ACCCGCGGCG
CTCCGCTCCC GGG

FIGURE 6H

MINT32 (SEQ ID NO:32)

CCCGGGTACC	TGCACAGCTC	GCTCCCTCCC	ATCCTTCGGG	TCTTCGCTCG	AACGTCCGCT
CCTCGGTGAG	GCCTTCCCTG	GACAACGCAT	TTGAAACGTA	ACCCCAAGGC	AAGAAGCCAC
CTTCCAGGCG	CGCAGCCGAA	GCCCAGTGCC	AAGGAGGCCG	GAGACTCGGG	TGCCCCGCGCA
TCCCGAAAAC	AGCCTCTGAG	GGGTCCTCTG	AGCATCCTTC	CAGCGTGTTT	GGGAGGCAAA
CTCGTTGACT	AGCTCTTGAG	AGGAGTGGCT	AGAGGAATCC	AGGCGGGGAA	GGGGACGGTG
GACTCCAGGA	GAGTGTAATT	TACAAAGGCG	GGGGGCGGGG	ACGCCCAGGT	CCGAGTCCCA
GGACTCTGCG	CCGGACGCTT	CGCCCCGCCCT	TTCAGGTCCC	CTGCCCCGGTC	CTCGTACCCG
CGCGGGTCCG	GAGAACCTCT	GAGCACCGGC	CCCCAGCCCC	CGGG	

MINT33 (SEQ ID NO:33)

CCCGGGCAGA	AAGGCTCGGA	TGGCGGTGGC	AGAAAGGCTC	GGAGGCGGTG	GCCTCAGATC
CCTGGTCCCT	CCGGGTCACT	GTCGGCTAAT	TCTGGGGGAA	GGACTGGGCA	AGGCTGTTTG
GAAGAAGTCA	GCGCCCGGG				

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25251

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 15, 19, 91.2, 91.52, 199, 320.1, 810; 536/23.1, 24.31, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97/45560 A1 (NORTH SHORE UNIVERSITY HOSPITAL RESEARCH CORPORATION) 04 December 1997 (04.12.97), see entire reference, especially pages 2-6, 14-15, 23-24, 28-29, 35.	1-4, 7-27, 29-38, 41-42, 45, 62-65
Y	ZRIHAN-LICHT et al. DNA methylation status of the Muc1 gene coding for a breast-cancer-associated protein. Int. J. Cancer. July 1995, Vol. 62, No. 3, pages 245-251, especially pages 246-259.	1-4, 7-27, 29-38, 41-42, 45, 62-65
Y	WO 96/35704 A1 (THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 14 November 1996 (14.11.96), see entire reference, especially pages 24, 32.	10, 21-22, 32-38, 41, 62-65

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 MARCH 2000

Date of mailing of the international search report

21 MAR 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231

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Authorized officer

DIANA JOHANNSEN

Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MERLO et al. 'DNA methylation and inactivation of tumor suppressor genes.' In: Hereditary Cancer, Second International Research Conference on Familial Cancer. Edited by HJ. Muller et al. Basel: Karger, 1996, pages 152-160, see entire reference, especially pages 153-157.	10, 21-22
Y	WEST et al. Hypomethylation of the amyloid precursor protein gene in the brain of an Alzheimer's disease patient. J. Mol. Neuroscience. 1995, Vol. 6, No. 2, pages 141-146, especially page 143.	26
A	AHUJA et al. Association between CpG island methylation and microsatellite instability in colorectal cancer. Proceedings of the American Association for Cancer Research. March 1997, Vol. 38, pages 360-361.	1-4, 7-27, 29-38, 41-42, 45, 62-65
A	US 5,786,146 A (HERMAN ET AL) 28 July 1998 (28/7/98), see entire reference.	1-4, 7-27, 29-38, 41-42, 45, 62-65
A, P	US 5,912,147 A (STOLER ET AL) 15 June 1999 (15/6/99), see entire reference.	1-4, 7-27, 29-38, 41-42, 45, 62-65.
A	US 5,501,964 A (WIGLER ET AL) 26 March 1996 (26/3/96), see entire reference.	1-4, 7-27, 29-38, 41
A	LISITSYN et al. Cloning the differences between two complex genomes. Science. 12 February 1993, Vol. 259, pages 946-951, see entire reference.	1-4, 7-27, 29-38, 41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25251

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 5-6, 28, 39-40, 43-44, 46-61
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

A sequence search could not be conducted due to errors in the computer-readable form of the sequence listing.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C12Q 1/44, 1/48, 1/68; C12P 19/34; C12N 9/22, 15/00; C07H 21/02, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/6, 15, 19, 91.2, 91.52, 199, 320.1, 810; 536/23.1, 24.31, 24.33

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

USPT, EPAB, JPAB, DWPI, MEDLINE, CANCERLIT, CAPLUS, LIFESCI, SCISEARCH, EMBASE, BIOSIS
search terms: CpG islands, methylation, restriction, ligation, adaptor, adapter, isoschizomer, SmaI, XmaI, PCR,
hybridization, cancer, carcinoma, tumor, malignancy, atherosclerosis, diabetes, dementia, representation difference
analysis, p16, Rb, VHL, hMLH1, BRCA1, chemotherapy, pharmaceutical

10/603, 138

6/23/03

Catherine

Lofton - Day et al.

Sequence Listing

Part 1

Sequence listing

<110> Cathy Lofton-Day
 Andrew Sledziewski
 Jorn Lewin
 Fabian Model
 Tamas Rujan

<120> Methods and nucleic acids for analyses of colorectal cell
 proliferative disorders.

<130> 47675-47

<160> 8475

<210> 1

<211> 2280

<212> DNA

<213> Homo Sapiens

<400> 1

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atgggtactt	attttacatt	cagatggaag	gcattattgg	atatgtatta	aaaaaaagac	180
cccctgaaaa	aaataaaata	aaataaaaaca	tcaccatcaa	aataaaaagaa	cccaaaacaa	240
cccctaaaaa	cttccctcaa	caaaatacat	tgtaaactca	taaaatggac	tgatgactag	300
ccatgcaaat	gtcctaaata	aaacctttac	atTTTTTTca	cagttaactt	atgctctgaa	360
ctgcctaccg	atcacaaata	atggcgaaat	ggcactttct	gattatactg	tatttttgtt	420
atagaaagtt	tgatacgatg	gaacttatca	ggtaagaggg	tgggtgctgt	gaacgagatg	480
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tcgtgcaact	gcccattccct	gcgtagccaa	cagggagcca	tcacggggct	aatccacagg	660
ggaaaaatag	atatctatct	ctctatatag	atgtggatat	atgtatatat	gtatagaacc	720
gcggcatcca	acccacacag	ccccggggcc	gagggcgagg	gcactgtcag	ttcttccagc	780
agggtcgtgc	tgggctttct	gtcaaaaggg	gctctcagca	aagagcgagc	tggctgcgct	840
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<213> Homo Sapiens

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<211> 286

<212> DNA

<213> Homo Sapiens

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<212> DNA

<213> Homo Sapiens

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<212> DNA

<213> Homo Sapiens

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<212> DNA

<213> Homo Sapiens

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 <212> DNA
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<212> DNA

<213> Homo Sapiens

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<211> 2398

<212> DNA

<213> Homo Sapiens

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<400> 48

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<212> DNA

<213> Homo Sapiens

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<213> Homo Sapiens

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<212> DNA

<213> Homo Sapiens

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<211> 2344

<212> DNA

<213> Homo Sapiens

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<212> DNA

<213> Homo Sapiens

<400> 54

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<211> 2264

<212> DNA

<213> Homo Sapiens

<400> 55

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<211> 1334

<212> DNA

<213> Homo Sapiens

<400> 56

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<211> 2501

<212> DNA

<213> Homo Sapiens

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<400> 58

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ttatgtaa	at	at	at	at	at	360
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<210> 307

<211> 2477

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 307

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<210> 308

<211> 3685

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 308

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<210> 309

<211> 3685

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 309

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<211> 2407

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2407

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 311

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<211> 2229

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 313

<211> 2229

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 6887

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<223> chemically treated genomic DNA (Homo sapiens)

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 319

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<211> 2265

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 320

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<211> 2265

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<220>

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<211> 4721

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2427

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<223> chemically treated genomic DNA (Homo sapiens)

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<211> 3093

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 3093

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2436

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 341

<211> 2264

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 341

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<211> 3104

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 3104

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 343

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<211> 2493

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2493

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 345

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<211> 2315

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 346

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<210> 347

<211> 2315

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 347

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<210> 348

<211> 2304

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 348

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<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 350

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<212> DNA

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 351

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<223> chemically treated genomic DNA (Homo sapiens)

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<223> chemically treated genomic DNA (Homo sapiens)

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 355

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 355

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<210> 356

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 356

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<210> 357

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 357

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 <211> 2470
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 358

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<210> 359
 <211> 2470
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 359

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<210> 360

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 360

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<210> 361

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 361

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<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 362

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<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 363

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<211> 947

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 364

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 367

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c g g c g g a g g a	c g g g a c g a g g	a t g g c g g a t c	g a a t t t g g t a	g a g g t t g g g g	t t c g t t g g g t	600
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g a c g g a g g g a	g g g a g c g a g c	g a a g g a t a t a	t t t a a g t a a g	g g g g g t t g t g	a t t a a g t a g t	720
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t c g c g g g g t g	t t t g t g t t t t	c g a g t t t t a t	t g g t t t t t g c	g t t t a g a g a c	g t a t t g g t t t	900
t t t t t t t t c g	t t g g t t t c g c	g g c g t t a g g a	t a g a g g a t g a	t c g a a t c g t a	a a a t t t t g t a	960
t t a t t g g g t t	t a g t t t t t g g	t t t t t t t t t t	t t c g t t a a a t	t t t t t t g a a t	t c g g t t g g a g	1020
t a a g a t t t t t	g g g t t t t a t a	g g a t g t t g g a	t a t t t t g g g a	g a g g a g t t g g	a g a g a g g g c g	1080
a g g t t t t g t t	t t g g g t g g c g	t a g t g t t t t c	g t g t t t a t a g	t g g g g a g t g a	g g g g t g g g g a	1140
g t t g g t g a a t	t t t t t a a g t g	g a a a g a g g g a	g a g t t t a t t t	t t t t t t t t t t	t a a t a a t a a g	1200
t t t a t a t g g t	t t g a a t g a a t	t t t a g t t t t t	t g g t a g a a t a	g t t t a g g g a a	a g t t a t t g a t	1260
g g t t t g t t a t	a g g g t t g g t a	t t t g g t t t a g	t t a a t t t a t g	t g t t t a a g t t	a g t g t g a a t g	1320
g g t a t t t t t t	t t t a t a t a t a	t g t t t a t t t a	t t t t t t t a a a	t a t t t t t t t a	t t c g t t t a t t	1380
t a t t t a t t t a	t t t a t t t t t t	t t t a t g t t t t	t t t g g g g t t t	t t a t a t t t a t	t t a t t t t t t t	1440
g t t t a t t t a t	t a a t t a t t t a	g g t t t a t t t a	t t a t t t t a t t	t a t t t a t t t a	t t t a a t t a t a	1500
t a t t t t t t a a	t t t a t t t a a t	t a t t t a t t t t	t t t a t t t a t t	t t t t t a t a t a	a t t t t t t a t t	1560
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t g t a t g g a t t	t t t t g t t t g g	t g a g t t a t a t	a g a a a a t g a a	a g t g a t t a t t	a g t g g t t g t g	2040
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t a a g a a a t t g	a a a a g t t g a g	a g a a a t g t a t	t t a g t t a t t t	t t t t a t g t a t	t t a t t t a t t a	2160
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<210> 370

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 370

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g g t t t a t t t t	a a t g t t t g g a	g t a t a t a t t t	t a a t g a a t a t	t t a t t t t a t t	t t a t t t t t t t	120
t t a t t t t t g a	a t t a a g t a a t	t t t g a a t t t a	a a g t t g t t a t	g a t t a g t a t t	g a a a a g a t t a	180
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g t t a t a t a t g	a a g g t t g a a g	t t t g a t t t t g	t t t t g t g a t t	a t t a t t t t a a	a t a t t t g a t g	300
a a a t t a a a t t	t t a g t g t t t g	g a a t g g t a g t	a t a a t a a a t t	t a t t a a g a a t	a a a t a a t t t a	360
t t g t a a a a a t	a t a t t g a t t t	t t a a a t g a t g	t a a t t g a t a g	t t a t a t t a t t	g t a g a g g g t t	420
g a t a a a t a a t	a a a a g a a a t g	a a a g a t g t a t	a t g g t g a g a a	t t g a a a t t a t	t t t g a t a a g t	480
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a t a a a a g a a a	t t t t a t a a a t	g c g t a t g t a t	a g g a g t t t a a	g t t a t t a a a a	g t t t t a a a g t	600
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g a g t t g t g a g	g a t a t t t t t a	t a t t t g t g t a	g a a g g a a a a g	t t t g t t a t t t	a t t t t a g t a t	780
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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 371

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<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 372

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<210> 373

<211> 3000

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 373

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<210> 374

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 374

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<210> 375

<211> 2501

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 376

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<210> 377

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 377

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<210> 378

<211> 1508

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 378

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gtttgtttat	tttttttttt	atatcgatta	gatgtaaacg	agtgttatta	aaagtatatt	240
ataggtat	atatagattt	tttttataag	ggatttataa	agttagatg	tgaaatgtat	300
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tacgttcggg	ttaatcgcca	gtagaataaa	tttttggtgg	gcggttagga	agtttttttt	780
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gaatgggttt	gggcggggag	gtgaagagaa	gttaggaatg	ttttatgttt	ttttaatgga	1140
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cggtaggttt	ttaagcggcg	cgcggtgttt	ttgtattttt	taaaattttt	gaaatatttt	1440
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<210> 379

<211> 1508

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 379

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atttgtcgtt	ttgggcgttt	ggcggagagt	ttcggggatt	gttttttagga	ttcgggtaaa	180
taatatTTTT	tattttgtaa	tttatttttt	cgaattttta	gtaggtaggg	tcggatcgcg	240
gggttttcgc	gataggggag	tatttttagat	ttatttagaa	aacgcgtttt	gggtttaata	300
gatttaattt	taggatttta	attttttttt	tcgtgttttt	ttagtttagg	ggttttcgag	360
gttttttttt	tattaggaaa	atataaaata	tttttggttt	ttttttattt	tttcgtttag	420
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aagtaggtat	gtagaattta	atattttttt	ttcgtttttg	aaatgttttt	taaacgtttc	1380
gaaataagaa	atgaagattt	tttaattttt	tataatttta	ttatcgatat	tttttggaat	1440
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<210> 380

<211> 286

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 380

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aaatgggatg	ttattttcgc	ggggttcgtt	tttcgcgagg	tgtttatttc	gtatttggtta	180
tgtaaaacga	gggagcgtta	ggaaggaatt	cgttttgtaa	agttattggg	tttggttatt	240
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<210> 381
 <211> 286
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 381

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ttgggggttta	gttttataaa	ttacggtggg	taatgttagt	tgattttatt	tttttagaga		240	
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<210> 382
 <211> 2501
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 382

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aggttgtagt	tattaaaatt	ttaggaaata	attttttatt	tttttaggtg	aaagggtttt	540
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tgagtttaga	gttttagttt	aatagtaatg	gattggaaga	ttagtttagg	ttttattaat	660
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agttaacggt	ttttgtagcg	cggcgggttc	gaagtcgtcg	ttcggagttg	tttttttttt	2220
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<210> 383

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 383

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agtcgattga	ggtagttgcg	ggagagaaga	cgggggaggg	ggggaaggta	gggtgggggg	180
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<210> 384

<211> 2448

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 384

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<210> 385

<211> 2448

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 385

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<210> 386

<211> 2344

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 386

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<210> 387

<211> 2344

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 387

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 388

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<210> 389

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<223> chemically treated genomic DNA (Homo sapiens)

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<210> 390

<211> 2206

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 390

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<210> 391

<211> 2206

<212> DNA

<213> Artificial Sequence

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<223> chemically treated genomic DNA (Homo sapiens)

<400> 391

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<211> 2233

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 392

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<211> 2233

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 393

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<211> 2398

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 394

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<210> 395

<211> 2398

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 395

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cg	cg	gc	gc	gt	tg	ag	gt	gc	gt	ta	at	300
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<210> 396

<211> 2114

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 396

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<210> 397

<211> 2114

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 397

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<210> 398

<211> 2382

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 398

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<210> 399

<211> 2382

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 399

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<211> 2192

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 400

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<211> 2192

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 401

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<210> 402

<211> 2192

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 402

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<211> 2192

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 403

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<211> 2244

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<222> (2126, 2128, 2131, 2132)

<223> unknown base

<400> 404

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<211> 2244

<212> DNA

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

<220>

<221> unsure

<222> (113, 114, 117, 119)

<223> unknown base

<400> 405

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<210> 406

<211> 2420

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 406

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<210> 407

<211> 2420

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 407

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<210> 408

<211> 2344

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 409

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<211> 2366

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 410

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<210> 411

<211> 2366

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 411

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<210> 412

<211> 2264

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 412

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<210> 413

<211> 2264

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 413

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<211> 1334

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 415

<211> 1334

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 415

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<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 416

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<212> DNA

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<223> chemically treated genomic DNA (Homo sapiens)

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<212> DNA

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<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2327

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2280

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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 <223> chemically treated genomic DNA (Homo sapiens)

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 <211> 2477
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

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<211> 2477

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 423

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<211> 3685

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 424

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<210> 425

<211> 3685

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 425

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<210> 426

<211> 2407

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 426

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<210> 427

<211> 2407

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 427

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<210> 428

<211> 2229

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 428

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<211> 2229

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 429

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<211> 6887

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 431
<211> 6887

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2820

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 434

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<210> 435

<211> 2820

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 435

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<210> 436

<211> 2265

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 436

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2265

<210> 437

<211> 2265

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 437

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<210> 438

<211> 5907

<212> DNA

<213> Artificial Sequence

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<223> chemically treated genomic DNA (Homo sapiens)

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<210> 444

<211> 2146

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 444

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<210> 445

<211> 2146

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 445

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<210> 446

<211> 2427

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 446

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<210> 447

<211> 2427

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 447

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<211> 3015

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 3015

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 449

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<211> 3093

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 3093

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2436

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 453

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<220>
 <223> chemically treated genomic DNA (Homo sapiens)

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<210> 458

<211> 3104

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 458

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<210> 459

<211> 3104

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 459

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<210> 460

<211> 2493

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 460

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<210> 461

<211> 2493

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 461

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<210> 462

<211> 2315

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 462

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<210> 463

<211> 2315

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 463

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<210> 464

<211> 2304

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 464

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<211> 2304

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 466

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<210> 467

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<213> Artificial Sequence

<220>

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<210> 472

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 472

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<210> 473

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 473

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<210> 474

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 474

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<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 475

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<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 476

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<210> 477
 <211> 2470
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 477

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<210> 478
 <211> 2470
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 478

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<210> 479

<211> 2470

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 479

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<210> 480

<211> 947

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 480

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<210> 481

<211> 947

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 481

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<210> 482

<211> 3120

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 482

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<211> 3120

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 483

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<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 484

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<210> 485

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 485

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<210> 486

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<212> DNA

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 486

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<210> 487

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 487

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<211> 3000

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 488

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<211> 3000

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 489

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<210> 490

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 490

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<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 491

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<210> 492

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 492

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<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 493

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<220>
 <223> chemically treated genomic DNA (Homo sapiens)

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<220>
 <223> chemically treated genomic DNA (Homo sapiens)

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<210> 496

<211> 286

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 496

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<210> 497

<211> 286

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 497

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<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 498

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47675-47.txt

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<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 499

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<210> 500

<211> 2448

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 500

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<211> 2448

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 501

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 <211> 2344
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

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<210> 503
 <211> 2344
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 503

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<210> 504

<211> 2350

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 504

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<210> 505

<211> 2350

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 505

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<210> 506

<211> 2206

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 506

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<210> 507

<211> 2206

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 508

<211> 2233

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 508

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<210> 509

<211> 2233

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 509

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<210> 510

<211> 2398

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 510

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<210> 511

<211> 2398

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 511

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 <211> 2114
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 512

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<210> 513
 <211> 2114
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 513

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<210> 514

<211> 2382

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 514

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<210> 515

<211> 2382

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 515

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<210> 516

<211> 2192

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 517

<211> 2192

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 517

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<210> 518

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<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 518

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<211> 2192

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 519

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<213> Artificial Sequence

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<220>

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<222> (2126, 2128, 2131, 2132)

<223> unknown base

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<211> 2244

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<220>

<221> unsure

<222> (113, 114, 117, 119)

<223> unknown base

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<211> 2420

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2420

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<223> chemically treated genomic DNA (Homo sapiens)

<400> 524

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<211> 2344

<212> DNA

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<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2366

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<223> chemically treated genomic DNA (Homo sapiens)

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<211> 2264

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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gagttagggg	gtgggggtgtg	gggggtgtgt	gatgtgtgat	gttggttttt	ggaagtgatg	2160
tgtttgtggg	gaaaaggtag	ggaggggggtg	gtgttttttag	ttggtttggg	gggtgtgttg	2220
tttggagatg	gaaagttttg	gagtttgagt	aggtttggtt	gtag		2264

<210> 529

<211> 2264

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 529

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tgggtgttatg	tgttattgtg	tttttgtgt	ttgtttttta	gtttatttag	ttgggtgggg	180
ttttgtggat	tagtgtagg	ggattttgt	gtgttgagat	atgttttttg	gggtatgggg	240
atagtgtttt	gtgggttttt	attatgtagg	tggaaaggga	taaatagggt	tatttgtatg	300
tgttttttga	agtgtgtttt	tttttttaa	gttttgggag	tttttgggat	aggaaatatg	360
gtaatttgtt	taggtagtgt	agaaataaag	agataaatgt	aatgtgtatt	tatattttta	420
agtttatttt	tttagtatgt	ataaattaaa	tatgtaatag	gtgtggggat	ttagttatat	480
ttagggtgaat	aaaatgtttt	tttttttgta	ttttaatgag	ggaagagaag	aaaattttta	540
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tgttttaaagg	agatattttt	tttttttttag	gttttttttt	tagttttta	gagattaatt	660
aaattgatag	aaaagtgtta	gatttgtaaa	aataaatttt	aatttttagt	attggtgaat	720
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gttttttaa	tgaagtaggt	agttaatagt	tgtggatgaa	taaatgaatt	ggataaattt	840
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gaattt	gaatttt	tagatt	attttg	ggaagag	ggatgat	1740
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gttttt	attaa	ttttta	agttata	gtttgt	tttgg	1860
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aggaagg	gttgatt	tttgt	ggagat	ttttat	aaaagt	2040
attgat	atttt	tgaat	ttaat	agttg	gttgg	2100
aatgg	ttttt	attgt	gtgtt	ggttt	attttt	2160
tttag	tgagt	ggatt	tgtat	tatgt	taattt	2220
atttt	gagat	tttatt	tggtt	ggtt		2264

<210> 530

<211> 1334

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 530

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gtttt	tagtttt	gtggat	ttttg	gggtt	ttagt	180
gatgt	aaatttt	gattat	gggtt	gttgt	tggtg	240
tattg	gttgt	ttgtt	tggtt	atgtt	gttgt	300
tggtt	gattt	ttttt	ttgtt	ttgt	gtatg	360
gttat	tgtgg	tttgt	tgtag	ggata	tgagg	420
tgttg	tgttg	tgttat	tttat	ttgtt	gaagt	480
gtttt	ttttt	tgatt	atgtt	gttgg	ttttg	540
tttag	ttgtt	ttttt	ttgt	atatg	ttagt	600
ttagt	ttagt	ttttt	tttat	attgt	atttt	660
ttatt	ttatt	aaata	aattg	ggttt	tgagg	720
ttttg	ggtag	gtagg	tagga	ttgt	gtgtt	780
tggag	aggatt	gaaat	atggg	ttttt	tattg	840
aaagag	aatttt	gaaat	aatag	ttgtt	taaag	900
agttt	ggatg	gaaat	tatga	aatga	aaag	960
attaa	gattg	gaatt	ttttt	agg	agg	1020
ggttt	tgaag	gttat	tttt	gttgg	aatag	1080
ttttt	ttttt	ttttt	atttt	ttttt	ttttt	1140
ttagg	aaag	gtttt	ttag	ttttt	gtttt	1200
gtttt	agtat	agttg	aaga	tgtgg	ttagg	1260
gtggg	ttgag	ttagg	tgtag	ttgag	aattt	1320
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<210> 531

<211> 1334

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 531

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tattattttg	aagtttaggg	aagtaaagag	aggggtatat	ttggattgta	aaattaatgt	180
tttttggtgt	ttaggagaga	agggaatgag	agagagagag	agatagagag	agagagagag	240
agagagagag	agagaaattt	tattgaaatt	tagttttttt	agaatttgtg	tgatttggtt	300
tttaatggga	gattagtgtg	attttatggg	atttttgtta	ggaattagtg	atttttttgt	360
agttattatt	tgatttattg	tttttttgtt	tatttttttt	tataaagtta	tttttttttt	420
attttagtaa	gatttttttt	tttaatgatg	ataaagtttt	tgtttttagt	tttttttttag	480
gattgggtgt	tttttaaaat	agtgaattta	gaaaattatt	ttgtttaata	ttttttaaaa	540
tttttgtagt	tttaatgtaa	gtgtaagtat	gtaaagggtt	tttggtatat	ttgtattttt	600
tgtttatttt	agaattattt	tttatttttg	ggtttgtaat	agtttttttt	gttttttttg	660
atagagggtg	gtggtattag	gggttttagg	tagtaggagg	tgaggggttg	aggagggtgt	720
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gaggtaggga	aagtagtgag	ttttattggg	tgggttatga	tgagtagtat	gatgggtagt	900
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taggaggaga	tgggagttta	ggggtagatg	agtggagttt	gaggaggtag	ggtggaggga	1320
gagttaaggt	gttt					1334

<210> 532

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 532

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ttttttttat	tttttaggtt	agttgatatt	tagggaaaat	aggaaaggat	ttatgtgaaa	180
tattgggggt	tgaatttttt	ttgataataa	gttttttaaa	aaagattttt	aaatgaaatt	240
tttttggttt	tttaatttta	tttttggttt	gggggtatat	gtgaagggtt	gttatataag	300
taaatttgtg	tttttggtgt	ttgttgtata	gaatatttta	ttatttaggt	attatgttga	360
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ttatagtgtt	tgttgttttt	ttgtttgtgt	tataagtttt	tattatttag	tttttattta	480
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aatgaagtt	ttaaagatat	aatataaata	ttatattttt	ttttattata	aaaatttttt	660
gttgaatttg	atttatattt	aattaatgag	ttttgtttta	tgaaagattt	tttggataaa	720
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ggttttattt	ataagtggga	gttgaataat	gagaatatat	ggttatatgg	tgggtgattaa	1020
tatatatttg	tgtttggtga	gtgggggtgt	ggggaggagg	agtattagga	agaatagtta	1080
agggatattg	ggtttaatat	ttgggtgatg	ggatgatttg	tatagtaaat	tattatggtg	1140
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gttggatggg	taggtgtggg	ggtttatgtt	tgtaatttta	gtatttttgg	gaagttgagg	1260
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ataataataa	ataaaaataa	ataaaaataa	ataaaaataa	ataaaaataa	ataaaaataa	1560
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gga	att	ttt	gtg	atg	1740
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ttt	ttt	gtt	gtt	ttt	1860
tgg	ggt	gtg	gtg	gtg	1920
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tag	ggt	gtg	gtg	gtg	2160
gat	ggt	gtg	gtg	gtg	2220
ggg	ggt	gtg	gtg	gtg	2280
ttt	ttt	gtt	gtt	gtt	2340
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<210> 533

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 533

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gtt	gtg	atg	gtt	gaa	aat	aa	agg	ggt	ggt	ggt	ggt	g	240
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ttt	ttt	gaaa	gtt	gtt	aat	gtt	gtt	ggt	ggt	ggt	ggt	g	360
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tgt	gtt	ggt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	480
agt	gtt	gtt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	540
ttg	gtt	gtt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	600
ggg	gtt	gtt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	660
tgt	gtt	gtt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	720
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gtg	gtt	gtt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	840
ggg	gtt	gtt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	900
gaa	gtt	gtt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	960
ttt	gtt	gtt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	1020
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gtg	gtt	gtt	ggt	ttt	ggt	ggt	ggt	ggt	ggt	ggt	ggt	g	1380
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tttta	aaagt	gggt	gtt	ttg	ag	ttt	at	atgt	ttt	gtg	atgt	2460
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<210> 534

<211> 2327

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 534

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ataaa	attgg	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	180
taggt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	240
ttttt	gtatg	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	300
ttttt	taggt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	360
gaaat	tatta	ggatg	ttttt	agatt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	420
tgagt	tattt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	ttttt	480
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tgaaa	attgaa	aaatt	ttgaa	aattt	ttgaa	gagtt	ttttt	tgaat	aaatt	taaat	ttttt	1140
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ggtag	gatgt	gttgg	attgg	gtttt	ttagg	gtttt	ttggg	ttttt	ttggg	ttttt	ttggg	1260
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<400> 1168
TTCGGGAAAG TAAAGCGTAT T

21

<210> 1169
<211> 24
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1169
CGTAAACTCC TCCTCTATAA CGTC

24

<210> 1170
<211> 30
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1170
AGAGGTCGAG TTTGTTATA GCGAAGGCGT

30

<210> 1171
<211> 24
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1171
CACGTAAACT CCTCCTCTAT AACG

24

<210> 1172
<211> 24
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1172
AAACGATAAT ATAACCTACT CCGC

24

<210> 1173
<211> 24
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1173
AACGATAATA TAACCTACTC CGCC

24

<210> 1174
<211> 23
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1174
ACGTAAACTC CTCCTCTATA ACG

23

<210> 1175
<211> 22
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1175
TCATAAACAA AACGTCTCGA AT

22

<210> 1176
<211> 24
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.

<400> 1176
CGTTATAGAG GAGGAGTTTA CGTG

24

<210> 1177
<211> 32
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1177
GGTGGCGGAG TAGGTTATAT TATCGTTTTG GG

32

<210> 1178
<211> 24
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1178
CATAAACAAA ACGTCTCGAA TTTA

24

<210> 1179
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1179
CGAAATCCTC ATAAACAAAA CG

22

<210> 1180
<211> 18
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1180
GTTTACGTGA TGGTGGCG

18

<210> 1181
<211> 26
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1181
ATCGTTTGG GTTCGGGGAG GGAGAG

26

<210> 1182
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<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1182
AGTTTACGTG ATGGTGGCG

19

<210> 1183
<211> 18
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1183
CGCCACCATC ACGTAAAC

18

<210> 1184
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1184
CTCATAAACA AAACGTCTCG AA

22

<210> 1185
<211> 25
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO 16.
<400> 1185
ACGTTATAGA GGAGGAGTTT ACGTG

25

<210> 1186
<211> 22

<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1186
AGTTTCGCGG TTTATAGAGG TT

22

<210> 1187
<211> 22
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1187
GAACGACCAA AACTAACTCG AA

22

<210> 1188
<211> 28
<212> DNA
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<220> bisulfite treated
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<400> 1188
GTCGCGTTGC GCGGTTTGAT TTAGTCGA

28

<210> 1189
<211> 22
<212> DNA
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<220> bisulfite treated
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<400> 1189
AACGATAATA CGAACGACCA AA

22

<210> 1190
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1190
GTTTCGCGGT TTATAGAGGT TT

22

<210> 1191
<211> 22
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GTAGTTTCGC GGTATATAGA GG

22

<210> 1192
<211> 22
<212> DNA
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<400> 1192
TAGTTTCGCG GTTATAGAG GT

22

<210> 1193
<211> 22
<212> DNA
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<400> 1193
ACGATAATAC GAACGACCAA AA

22

<210> 1194
<211> 22
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1194
CGATAATACG AACGACCAAA AC

22

<210> 1195
<211> 22
<212> DNA
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<220> bisulfite treated
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<400> 1195
GGTAGTTTCG CGGTTATAG AG

22

<210> 1196
<211> 22
<212> DNA
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<400> 1196
GTTTAGGTAG TTTCGCGGTT TA

22

<210> 1197
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
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<400> 1197
GAACGATAAT ACGAACGACC AA

22

<210> 1198
<211> 22
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1198
ACGACCAAAA CTAACGCGAA AT

22

<210> 1199
<211> 21
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1199
TTTCGCGGTT TATAGAGGTT T

21

<210> 1200
<211> 21
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1200
TAGTAGGGTT TCGATTTTCG G

21

<210> 1201
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
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<400> 1201
TACACCTATC TATAACGCG CC

22

<210> 1202
<211> 26
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
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<400> 1202
TGGTGGTTGG TGAACGTGC GATTGT

26

<210> 1203
<211> 23
<212> DNA
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<220> bisulfite treated
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<400> 1203
AGGTAGTTTC GCGGTTTATA GAG

23

<210> 1204
<211> 23
<212> DNA
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<400> 1204
AACGACCAAA ACTAACTCGA AAT

23

<210> 1205
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
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<400> 1205
TTAGTAGGGT TTCGATTTTC GG

22

<210> 1206
<211> 21
<212> DNA
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<400> 1206
TTCGCGGTTT ATAGAGGTTT C

21

<210> 1207
<211> 21
<212> DNA
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<400> 1207
CGATAATACG AACGACCAAA A

21

<210> 1208
<211> 22
<212> DNA
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<400> 1208
CGGTTTATAG AGGTTTCGGT TC

22

<210> 1209
<211> 21
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<400> 1209
GTTTCGCGGT TTATAGAGGT T

21

<210> 1210
<211> 21
<212> DNA
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<400> 1210
AACGATAATA CGAACGACCA A

21

<210> 1211
<211> 22
<212> DNA
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<400> 1211
TTTAGGTAGT TTCGCGGTTT AT

22

<210> 1212
<211> 21
<212> DNA
<213> Artificial Sequence

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<400> 1212
GAACGATAAT ACGAACGACC A

21

<210> 1213
<211> 22
<212> DNA
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<400> 1213
GTTTATAGAG GTTTCGGTTC GC

22

<210> 1214
<211> 22
<212> DNA
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<400> 1214
TTTCGCGGTT TATAGAGGTT TC

22

<210> 1215
<211> 21
<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1215

ACGATAATAC GAACGACCAA A

21

<210> 1216

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1216

TTAGTTTTGT ATCGTTCGGG TT

22

<210> 1217

<211> 21

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1217

TCCCTCGATA AATCAAAAAC G

21

<210> 1218

<211> 47

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1218

ATCGTTTTGG CGCGTTTATA GATAGGTGTA TGAAGATTTT TACGATT

47

<210> 1219

<211> 21

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1219

TTTATAGAGG TTTCGGTTCG C

21

<210> 1220

<211> 22
<212> DNA
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<400> 1220
TACGAACGAC CAAAACTAAC TC

22

<210> 1221
<211> 20
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<400> 1221
AGTAGGGTTT CGATTTTCGG

20

<210> 1222
<211> 21
<212> DNA
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<220> bisulfite treated
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<400> 1222
ACACCTATCT ATAAACGCGC C

21

<210> 1223
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<400> 1223
TAGGTAGTTT CGCGGTTTAT AGAG

24

<210> 1224
<211> 24
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<400> 1224
AATACGAACG ACCAAAATA ACTC

24

<210> 1225
<211> 21
<212> DNA
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<400> 1225
GCGGTTTATA GAGGTTTCGG T

21

<210> 1226
<211> 22
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1226
AACTAACTCG AAATCCCGAA CT

22

<210> 1227
<211> 23
<212> DNA
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<400> 1227
ATACACCTAT CTATAAACGC GCC

23

<210> 1228
<211> 21
<212> DNA
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<400> 1228
ACCTATCTAT AAACGCGCCA A

21

<210> 1229
<211> 23
<212> DNA
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<400> 1229
ATACGAACGA CCAAACTAA CTC

23

<210> 1230
<211> 21
<212> DNA
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<400> 1230
AGTTTCGCGG TTTATAGAGG T

21

<210> 1231
<211> 23
<212> DNA
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<400> 1231
AATACGAACG ACCAAAATA ACT

23

<210> 1232
<211> 21
<212> DNA
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<400> 1232
CGAACGACCA AACTAACTC G

21

<210> 1233
<211> 20
<212> DNA
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<220> bisulfite treated
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<400> 1233
CCTATCTATA AACGCGCCAA

20

<210> 1234
<211> 22
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1234

GATTTTCGAGT TAGTTTTGGT CG

22

<210> 1235
<211> 20
<212> DNA
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<400> 1235
TAACTTCTAT TACCCGCGCC

20

<210> 1236
<211> 24
<212> DNA
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<400> 1236
TTCGCGCGGG CGTATCGAGT TCGG

24

<210> 1237
<211> 20
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1237
CGAAATCCCG AACTACAAA

20

<210> 1238
<211> 20
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1238
GGGGGAGTCG CGTTGCGCGG

20

<210> 1239
<211> 21
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1239
CGACCAAAAC TAACTCGAAA T

21

<210> 1240
<211> 23
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1240
TTAGGTAGTT TCGCGGTTTA TAG

23

<210> 1241
<211> 20
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1241
AGGTTTAGGT AGTTTCGCGG

20

<210> 1242
<211> 21
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1242
CGTTAAGAGG CGAGGAAATA A

21

<210> 1243
<211> 21
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<213> Artificial Sequence

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<400> 1243
CCTAACTCGA CGCAACTAAC C

21

<210> 1244
<211> 25
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1244
AACGGCGGGC GGGACGAACG ATTAG

25

<210> 1245
<211> 20
<212> DNA
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<400> 1245
GCGGTTTATA GAGGTTTCGG

20

<210> 1246
<211> 22
<212> DNA
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<400> 1246
TAACTCGAAA TCCCGAACTA CA

22

<210> 1247
<211> 20
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1247
TCGCGGTTTA TAGAGGTTTC

20

<210> 1248
<211> 20
<212> DNA
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<400> 1248
CCCTAACTCG ACGCAACTAA

20

<210> 1249
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<212> DNA
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<400> 1249
GGTTTAGGTA GTTTCGCGGT

20

<210> 1250
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<400> 1250
TCGAAATCCC GAACTACAAA A

21

<210> 1251
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<212> DNA
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<400> 1251
GATAATACGA ACGACCAAAA CT

22

<210> 1252
<211> 22
<212> DNA
<213> Artificial Sequence

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<400> 1252
TAGTCGAGGT TTTGTAGTTC GG

22

<210> 1253
<211> 21
<212> DNA
<213> Artificial Sequence

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<400> 1253
ACGAACGACC AAAACTAACT C

21

<210> 1254
<211> 24
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1254
GATAATACGA ACGACCAAAA CTAA

24

<210> 1255
<211> 25
<212> DNA
<213> Artificial Sequence

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<400> 1255
TAATACGAAC GACCAAAACT AACTC

25

<210> 1256
<211> 20
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1256
CACCTATCTA TAAACGCGCC

20

<210> 1257
<211> 20
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<400> 1257
TTCGCGGTTT ATAGAGGTTT

20

<210> 1258
<211> 21
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<400> 1258
CGGTTTATAG AGGTTTCGGT T

21

<210> 1259
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<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1259
 CTCGAAATCC CGAACTACAA

20

<210> 1260
 <211> 22
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 <400> 1260
 CGTTAAGAGG CGAGGAAATA AG

22

<210> 1261
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1261
 CTAACCGAAA ACGCGAACT A

21

<210> 1262
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1262
 GTCGGGAGAG GGGCGCGGAA CGG

23

<210> 1263
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1263
 TAACCGAAAA CGCGAACTA

20

<210> 1264
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1264
GTTTCGGGATT TCGAGTTAGT TT

22

<210> 1265
<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1265
TTATAGAGGT TTCGGTTCGC

20

<210> 1266
<211> 21
<212> DNA
<213> Artificial Sequence

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<400> 1266
TTTAGGTAGT TTCGCGGTTT A

21

<210> 1267
<211> 21
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1267
GAGTTAGTGC GTTCGATTGT G

21

<210> 1268
<211> 20
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1268
GCCTACTCTA CTTCCGCGAT

20

<210> 1269
<211> 21
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1269
GGGGGCGGAA AGAGGCGCGT T

21

<210> 1270
<211> 24
<212> DNA
<213> Artificial Sequence

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<400> 1270
TAATACGAAC GACCAAACT AACT

24

<210> 1271
<211> 22
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1271
ATAAAGGTTT CGTTAAGAGG CG

22

<210> 1272
<211> 19
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1272
ATACCCCTAA CTCGACGCA

19

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<400> 1273
TTTAGTTTTG TATCGTTCGG GTT

23

<210> 1274
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<400> 1274
CGCGGTTTAT AGAGGTTTCG

20

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GTCGGAAGGT GCGAGTTAGT

20

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GATCGACCGA ATAAAAACAA AT

22

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<400> 1277
CGTTTTTAGA TCGTGATTTT CG

22

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1278

ACTAACTCGC ACCTTCCGAC

20

<210> 1279

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1279

AAGTTTTCGCG GCGGGGCGC GT

22

<210> 1280

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

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ATTTCGAGTT AGTTTGGTC GT

22

<210> 1281

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TCGGAAGGTG CGAGTTAGT

19

<210> 1282

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1282

GTAGGGTTTC GATTTTCGG

19

<210> 1283

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AACTTCTATT ACCCGCGCC

19

<210> 1284
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<400> 1284
ATCGTTCGCG CGGGCGTATC GAG

23

<210> 1285
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CAATCGAACG CACTAACTCG

20

<210> 1286
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ACCGAAAACG CGAAACTAAT

20

<210> 1287
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<400> 1287
AAGGTGCGAG TTAGTGCGT

19

<210> 1288
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<400> 1288
AGGTGCGAGT TAGTGCGTT

19

<210> 1289
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<400> 1289
TAGTTTTGTA TCGTTCGGGT T

21

<210> 1290
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<400> 1290
CTCGATAAAT CAAAAACGAA AA

22

<210> 1291
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<400> 1291
ACGCACTAAC TCGCACCTT

19

<210> 1292
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<400> 1292
AACGCACTAA CTCGCACCT

19

<210> 1293
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<400> 1293
TCGGGATTTC GAGTTAGTTT T

21

<210> 1294
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<400> 1294
TTCGGGATTT CGAGTTAGTT T

21

<210> 1295
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<400> 1295
TCGTTAAGAG GCGAGGAAAT A

21

<210> 1296
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<400> 1296
AGTTTCGCGG TTTATAGAGG TTT

23

<210> 1297
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<400> 1297

AACCGCGATA CCCCTAACT

19

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<400> 1298
AACTCGAAAT CCCGAACTAC A

21

<210> 1299
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<400> 1299
CGGTTTATAG AGGTTTCGGT

20

<210> 1300
<211> 20
<212> DNA
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<400> 1300
GAAACTAATC GTTCGTCCCG

20

<210> 1301
<211> 19
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<400> 1301
CGGGAAAGTA GCGGTAAGT

19

<210> 1302
<211> 22
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1302
CCGAACCTACA AAACCTCGAC TA

22

<210> 1303
<211> 28
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1303
AGATGCGAGG TTTAGGTAGT TTCGCGGT

28

<210> 1304
<211> 20
<212> DNA
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<400> 1304
AGGCGAGGAA ATAAGAGTCG

20

<210> 1305
<211> 21
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1305
CCCTAACTCG ACGCAACTAA C

21

<210> 1306
<211> 23
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1306
TTTAGTCGAG GTTTTGTAGT TCG

23

<210> 1307
<211> 33
<212> DNA
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<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1307
 TAGTTTGGT CGTTCGTATT ATCGTTCGCG CGG

33

<210> 1308
 <211> 20
 <212> DNA
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<220> bisulfite treated
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 <400> 1308
 AGGTTTCGTT AAGAGGCGAG

20

<210> 1309
 <211> 21
 <212> DNA
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 <400> 1309
 ACTAACTCGA AATCCCGAAC T

21

<210> 1310
 <211> 22
 <212> DNA
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 <400> 1310
 AGTTCGGGAT TTCGAGTTAG TT

22

<210> 1311
 <211> 18
 <212> DNA
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 <400> 1311
 ACTTCTATTA CCCGCGCC

18

<210> 1312
 <211> 21

<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1312
AGTCGAGGTT TTGTAGTTCG G

21

<210> 1313
<211> 19
<212> DNA
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<400> 1313
CCGAAAACGC GAAACTAAT

19

<210> 1314
<211> 25
<212> DNA
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<400> 1314
ATAATACGAA CGACCAAAC TAACT

25

<210> 1315
<211> 19
<212> DNA
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<400> 1315
GCGATACCCC TAACTCGAC

19

<210> 1316
<211> 20
<212> DNA
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<400> 1316
ACGATAATAC GAACGACCAA

20

<210> 1317
<211> 21
<212> DNA
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<400> 1317
ACTCGAAATC CCGAACTACA A

21

<210> 1318
<211> 20
<212> DNA
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<400> 1318
ACTAACCGAA AACGCGAAAC

20

<210> 1319
<211> 20
<212> DNA
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<400> 1319
TCGAAATCCC GAACTACAAA

20

<210> 1320
<211> 22
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<400> 1320
GGGTCGTTTT TAGATCGTGA TT

22

<210> 1321
<211> 19
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<400> 1321
CGGGTCGTTT TTAGATCGT

19

<210> 1322
<211> 21
<212> DNA
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<400> 1322
CACAATCGAA CGCACTAACT C

21

<210> 1323
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
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<400> 1323
TAAAGGTTTC GTTAAGAGGC G

21

<210> 1324
<211> 19
<212> DNA
<213> Artificial Sequence

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<400> 1324
TGTTTCGGTTT CGGAGGTAG

19

<210> 1325
<211> 21
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<400> 1325
ATCACGATCT AAAAACGACC C

21

<210> 1326
<211> 33
<212> DNA
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<220> bisulfite treated
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<400> 1326
GGCGTTGGAG CGGTTAGTGG TAGTCGGGTA CGG

33

<210> 1327
<211> 22
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1327
CGGTTTGATT TAGTCGAGGT TT

22

<210> 1328
<211> 40
<212> DNA
<213> Artificial Sequence

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<400> 1328
TTCGGGATTT CGAGTTAGTT TTGGTCGTTC GTATTATCGT

40

<210> 1329
<211> 21
<212> DNA
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<400> 1329
AAGGTTTCGT TAAGAGGCGA G

21

<210> 1330
<211> 19
<212> DNA
<213> Artificial Sequence

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<400> 1330
CTAACTCGCA CCTTCCGAC

19

<210> 1331
<211> 20
<212> DNA
<213> Artificial Sequence

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<400> 1331
TCGTTAAGAG GCGAGGAAAT

20

<210> 1332
<211> 19
<212> DNA
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<400> 1332
ATCGAACGCA CTAACTCGC

19

<210> 1333
<211> 21
<212> DNA
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<400> 1333
GTTTTGTAGT TCGGGATTTC G

21

<210> 1334
<211> 19
<212> DNA
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<400> 1334
AACTAACCGA AAACGCGAA

19

<210> 1335
<211> 19
<212> DNA
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<400> 1335
AACCGAAAAC GCGAAACTA

19

<210> 1336
<211> 22
<212> DNA
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<400> 1336
TCTCGAAAAA CTCTCCTAAA CG

22

<210> 1337
<211> 22
<212> DNA
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<400> 1337
CGTAAAGTTC GGTTTAATTT GG

22

<210> 1338
<211> 26
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<400> 1338
AGGCGTTATC GTCGGCGTAT TTCGGA

26

<210> 1339
<211> 22
<212> DNA
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<400> 1339
CTCGAAAAAC TCTCCTAAAC GA

22

<210> 1340
<211> 22
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<220> bisulfite treated
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<400> 1340
TCGAAAAACT CTCCTAAACG AC

22

<210> 1341
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<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1341

ACACCGCGAC TATTATACGA CT

22

<210> 1342

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1342

TTCGGTTGTT ATTGATCGTT TT

22

<210> 1343

<211> 33

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1343

ATTCGTTAGT TGTTTTCGGA GTCGAGTAGG TTT

33

<210> 1344

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1344

GAGTTAGTTA GGTTCGTAGG CG

22

<210> 1345

<211> 31

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<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1345

ATCGTCGGCG TATTTTCGGAG AAGAAGTTTT T

31

<210> 1346

<211> 21
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<220> bisulfite treated
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<400> 1346
TCGTGTTTCGG TTGTTATTGA T

21

<210> 1347
<211> 21
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<400> 1347
GTTCGTGTTC GGTGTTATT G

21

<210> 1348
<211> 22
<212> DNA
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<400> 1348
CGAAAACTC TCCTAAACGA CT

22

<210> 1349
<211> 23
<212> DNA
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<220> bisulfite treated
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<400> 1349
GAGTTAGTTA GGTTCGTAGG CGT

23

<210> 1350
<211> 22
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<400> 1350
CACCGCGACT ATTATACGAC TA

22

<210> 1351
<211> 22
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<400> 1351
TTCGTGTTTC GTTGTTATTG AT

22

<210> 1352
<211> 22
<212> DNA
<213> Artificial Sequence

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<400> 1352
TGTTTCGGTTG TTATTGATCG TT

22

<210> 1353
<211> 22
<212> DNA
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<400> 1353
CTTAATAAAA CCTCGATTTC CG

22

<210> 1354
<211> 22
<212> DNA
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<400> 1354
TTTGTTTATA GGCGCGTTAG AG

22

<210> 1355
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<400> 1355
GGTTCGAGCG GTGTAGGGTT GGGT

24

<210> 1356
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<212> DNA
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<400> 1356
TTCGTAAAGT TCGGTTTAAT TTG

23

<210> 1357
<211> 22
<212> DNA
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<400> 1357
AAACACCGCG ACTATTATAC GA

22

<210> 1358
<211> 22
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1358
AGTTAGTTAG GTTCGTAGGC GT

22

<210> 1359
<211> 23
<212> DNA
<213> Artificial Sequence

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<400> 1359
ACTTAATAAA ACCTCGATTT CCG

23

<210> 1360
<211> 21
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<400> 1360

GTGTTTCGGTT GTTATTGATC G

21

<210> 1361
<211> 22
<212> DNA
<213> Artificial Sequence

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<400> 1361
TAAAACGATC AATAACAACC GA

22

<210> 1362
<211> 22
<212> DNA
<213> Artificial Sequence

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<400> 1362
GTATTTTTCG GTAAGAGACG GC

22

<210> 1363
<211> 34
<212> DNA
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<400> 1363
TTCGGGAGTT ACGGTTTGAG GGCGATTCGG GCGA

34

<210> 1364
<211> 22
<212> DNA
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<400> 1364
TCGTAAAGTT CGGTTTAATT TG

22

<210> 1365
<211> 21
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1365
TCGGTTGTTA TTGATCGTTT T

21

<210> 1366
<211> 23
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<400> 1366
ACACCGCGAC TATTATACGA CTA

23

<210> 1367
<211> 22
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1367
CGAACGACCA ATAAAAACAT AA

22

<210> 1368
<211> 22
<212> DNA
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<220> bisulfite treated
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<400> 1368
GCGTATTTTCG GAGAAGAAGT TT

22

<210> 1369
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<400> 1369
TTCGAGAAAG ATGTCGTCGT TTTAATAGTA ATTTATTTTC GT

42

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<212> DNA
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<400> 1370
TATTTTTCGG TAAGAGACGG C

21

<210> 1371
<211> 20
<212> DNA
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<400> 1371
AATTTCCT TACCCGACCG

20

<210> 1372
<211> 22
<212> DNA
<213> Artificial Sequence

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<400> 1372
TCGTTTAGGA GAGTTTTTCG AG

22

<210> 1373
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<212> DNA
<213> Artificial Sequence

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<400> 1373
AAAGATGTCG TCGTTTAAAT AGTAATTAT TTTCGTTTCG GAG

43

<210> 1374
<211> 23
<212> DNA
<213> Artificial Sequence

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<400> 1374
ACGAACGACC AATAAAAACA TAA

23

<210> 1375
<211> 22

<212> DNA
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<400> 1375
TTAAAACGAT CAATAACAAC CG

22

<210> 1376
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
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<400> 1376
ATTTATCCTT ACCCGACCGA C

21

<210> 1377
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TATTTGTTTA TAGGCGCGTT AG

22

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<211> 23
<212> DNA
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<400> 1378
GTTCGGTTGT TATTGATCGT TTT

23

<210> 1379
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GTTAAAACGA TCAATAACAA CCG

23

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<211> 21
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TTGTTTATAG GCGCGTTAGA G

21

<210> 1381
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ACTCTAATCG CTCGCACTAC C

21

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<400> 1382
ATTAAGTTTT ATGGTCGCGG TT

22

<210> 1383
<211> 41
<212> DNA
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<400> 1383
AGGGTGAGTC GAGATTTAGA CGTAGATTTT TTAATTTTGT T

41

<210> 1384
<211> 21
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<400> 1384
AACACCGCGA CTATTATACG A

21

<210> 1385
<211> 22
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<400> 1385
CCGCGACTAT TATACGACTA AA

22

<210> 1386
<211> 20
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<400> 1386
GACCAACTAC GTCGAACCAA

20

<210> 1387
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<400> 1387
GTTCGGTTGT TATTGATCGT TT

22

<210> 1388
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<400> 1388
CGTTCGTGTT CGGTTGTTAT

20

<210> 1389
<211> 23
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<213> Artificial Sequence

<220> bisulfite treated
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<400> 1389
AACACCGCGA CTATTATACG ACT

23

<210> 1390
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<400> 1390
ACGAACCTAA CCGTAAAAAC G

21

<210> 1391
<211> 23
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<220> bisulfite treated
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<400> 1391
TTGGGTCGTA AGTTTATAG TCG

23

<210> 1392
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TACGTTTAT TAATTATTAG TCGGGGTTTCG GAAGCGCGTT CGG

43

<210> 1393
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<212> DNA
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<400> 1393
ACCGCGACTA TTATACGACT AAA

23

<210> 1394
<211> 20
<212> DNA
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<220> bisulfite treated
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<400> 1394
ACCAACTACG TCGAACCAAA

20

<210> 1395
<211> 22
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<400> 1395
TCGGTTGTTA TTGATCGTTT TA

22

<210> 1396
<211> 23
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1396
ATTTGTTTAT AGGCGCGTTA GAG

23

<210> 1397
<211> 21
<212> DNA
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<400> 1397
AATTAAGTTT TATGGTCGCG G

21

<210> 1398
<211> 22
<212> DNA
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<220> bisulfite treated
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<400> 1398
CAACTCTAAT CGCTCGCACT AC

22

<210> 1399
<211> 21
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<400> 1399
TAAGTTTTAT GGTGCGGTT T

21

<210> 1400
<211> 21
<212> DNA
<213> Artificial Sequence

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<400> 1400
TTAAGTTTTA TGGTCGCGGT T

21

<210> 1401
<211> 22
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<400> 1401
TATATTTGTT TATAGGCGCG TT

22

<210> 1402
<211> 21
<212> DNA
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<400> 1402
AAAACGATCA ATAACAACCG A

21

<210> 1403
<211> 23
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
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<400> 1403
ATATTTGTTT ATAGGCGCGT TAG

23

<210> 1404
<211> 20
<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1404

ATTTTTCGGT AAGAGACGGC

20

<210> 1405

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1405

AACCGAAACT TTACAACCCG

20

<210> 1406

<211> 22

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1406

ATTTTGTGTTG TTCGCGTTAA GT

22

<210> 1407

<211> 20

<212> DNA

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1407

GGTGCGTTTCG CGCGGACGGT

20

<210> 1408

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1408

TTTATCCTTA CCCGACCGAC

20

<210> 1409

<211> 22
<212> DNA
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<400> 1409
TGGGTCGTAA GTTTTATAGT CG

22

<210> 1410
<211> 22
<212> DNA
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<400> 1410
GTTTTATGGT CGCGGTTTTT AG

22

<210> 1411
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<212> DNA
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<400> 1411
TTCGCGTTAA GTCGGGTTTCG GTGCGT

26

<210> 1412
<211> 22
<212> DNA
<213> Artificial Sequence

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<400> 1412
AGTTTTATGG TCGCGGTTTT TA

22

<210> 1413
<211> 22
<212> DNA
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<400> 1413
AACGAACGAC CAATAAAAAC AT

22

<210> 1414
 <211> 21
 <212> DNA
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<220> bisulfite treated
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 <400> 1414
 GGCGTATTTC GGAGAAGAAG T

21

<210> 1415
 <211> 22
 <212> DNA
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 <400> 1415
 AGTCGTTTAG GAGAGTTTTT CG

22

<210> 1416
 <211> 22
 <212> DNA
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 <400> 1416
 ACTCGACTCC GAAAACAACT AA

22

<210> 1417
 <211> 19
 <212> DNA
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 <400> 1417
 GCGTTTCGTT CGTAAGGTT

19

<210> 1418
 <211> 45
 <212> DNA
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 <400> 1418
 TTCGTGTTTC GTTGTTATTG ATCGTTTTAA CGTCGTTAGG GACGA

45

<210> 1419
<211> 20
<212> DNA
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<400> 1419
CTCTAATCGC TCGCACTACC

20

<210> 1420
<211> 19
<212> DNA
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<400> 1420
TCCGAACCCC GACTAATAA

19

<210> 1421
<211> 22
<212> DNA
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<400> 1421
TAGTAATTTT GTTTCGGGTC GT

22

<210> 1422
<211> 21
<212> DNA
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<400> 1422
CTCGAACCAA CTCTAATCGC T

21

<210> 1423
<211> 23
<212> DNA
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<400> 1423

CGACTATAAA ACTTACGACC CAA

23

<210> 1424
<211> 21
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<400> 1424
CGGAAAAGCG GTGTTTATTA G

21

<210> 1425
<211> 23
<212> DNA
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<400> 1425
AGCGGTGAAG GGTTCGAGCG GTG

23

<210> 1426
<211> 20
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<400> 1426
TTTATGGTCG CGGTTTTTAG

20

<210> 1427
<211> 21
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<213> Artificial Sequence

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<400> 1427
TTTTATGGTC GCGGTTTTTA G

21

<210> 1428
<211> 22
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1428
AATTTTGTGTT GTTCGCGTTA AG

22

<210> 1429
<211> 22
<212> DNA
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<400> 1429
CTACTCGACT CCGAAAACAA CT

22

<210> 1430
<211> 21
<212> DNA
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<400> 1430
TGTTTATAGG CGCGTTAGAG C

21

<210> 1431
<211> 19
<212> DNA
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<400> 1431
ACCGAAACTT TACAACCCG

19

<210> 1432
<211> 22
<212> DNA
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<400> 1432
GAATTAAGTT TTATGGTCGC GG

22

<210> 1433
<211> 22
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<220> bisulfite treated
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<400> 1433
TTAAGTTTTA TGGTCGCGGT TT

22

<210> 1434
<211> 41
<212> DNA
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<400> 1434
GGGTGAGTCG AGATTAGAC GTAGATTTT TAATTTTGT T

41

<210> 1435
<211> 21
<212> DNA
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<400> 1435
TTAATAAAAC CTCGATTTC G

21

<210> 1436
<211> 21
<212> DNA
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<400> 1436
CGAAACCTCG AACCAACTCT A

21

<210> 1437
<211> 22
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<400> 1437
AAACTTCTTC TCCGAAATAC GC

22

<210> 1438
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<212> DNA
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<400> 1438
GTCGAGGTTT TTGTAGGTCG

20

<210> 1439
<211> 33
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<400> 1439
TTTGGAGTTA GTTAGGTTCG TAGGCGTTAT CGT

33

<210> 1440
<211> 22
<212> DNA
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<400> 1440
AACTCTAATC GCTCGCACTA CC

22

<210> 1441
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<400> 1441
CGTTCGTGTT CGGTTGTTAT T

21

<210> 1442
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<400> 1442
TTCCGAACCC CGACTAATAA

20

<210> 1443
<211> 21
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<400> 1443
AGTTAGTTAG GTTCGTAGGC G

21

<210> 1444
<211> 20
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<400> 1444
AACCTAACCG TAAAAACGCC

20

<210> 1445
<211> 23
<212> DNA
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<220> bisulfite treated
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<400> 1445
GTCGTTTAGG AGAGTTTTTC GAG

23

<210> 1446
<211> 19
<212> DNA
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<400> 1446
TTATCCTTAC CCGACCGAC

19

<210> 1447
<211> 24
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1447
AACTTAATAA AACCTCGATT TCCG

24

<210> 1448
 <211> 19
 <212> DNA
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 <400> 1448
 TTTTTCGGTA AGAGACGGC

19

<210> 1449
 <211> 21
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 <400> 1449
 TCGAAAACT CTCCTAAACG A

21

<210> 1450
 <211> 19
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<220> bisulfite treated
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 <400> 1450
 TCGGGAGTTA CGGTTTGAG

19

<210> 1451
 <211> 23
 <212> DNA
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<220> bisulfite treated
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 <400> 1451
 ACGACTATAA AACTTACGAC CCA

23

<210> 1452
 <211> 25
 <212> DNA
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 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1452
 GGTGAAGGGT TCGAGCGGTG TAGGG

25

<210> 1453
<211> 27
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1453
GGAGTTACGG TTTGAGGGCG ATTCGGG

27

<210> 1454
<211> 21
<212> DNA
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<400> 1454
CGAAAACTC TCCTAAACGA C

21

<210> 1455
<211> 20
<212> DNA
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<400> 1455
AAGTTTTATG GTCGCGGTTT

20

<210> 1456
<211> 20
<212> DNA
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<400> 1456
CGACCTACAA AACCTCGAC

20

<210> 1457
<211> 22
<212> DNA
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<400> 1457
GTAGTGCGAG CGATTAGAGT TG

22

<210> 1458
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<212> DNA
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<400> 1458
TTCGGTTGGG TTAAGTCGCG TAGCGCGG

28

<210> 1459
<211> 21
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1459
ACCAACTACG TCGAACCAAA A

21

<210> 1460
<211> 21
<212> DNA
<213> Artificial Sequence

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<400> 1460
CCGAAATCGC AATCTAAAAC T

21

<210> 1461
<211> 23
<212> DNA
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1461
AAAACACCGC GACTATTATA CGA

23

<210> 1462
<211> 20
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1462
GTTTATAGGC GCGTTAGAGC

20

<210> 1463
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1463
TTTTGTTGTT CGCGTTAAGT C

21

<210> 1464
<211> 21
<212> DNA
<213> Artificial Sequence

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<400> 1464
AACTTCTTCT CCGAAATACG C

21

<210> 1465
<211> 21
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1465
TAGTGCGAGC GATTAGAGTT G

21

<210> 1466
<211> 19
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<213> Artificial Sequence.

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1466
CCGAAACTTT ACAACCCGA

19

<210> 1467
<211> 19
<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1467

CGTTATCGTC GGCGTATTT

19

<210> 1468

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1468

TTTTATTCCC CGCTAACGAC

20

<210> 1469

<211> 22

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1469

TTTCGGTAAA TTCGTAGGTT TT

22

<210> 1470

<211> 37

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1470

TACGATGGCG GGGCGGCGGC GCGAATATAT AAAGGAG

37

<210> 1471

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1471

CCAACTACGT CGAACCAAAA

20

<210> 1472

<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1472
CGTGTTCGGT TGTATTGAT

20

<210> 1473
<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1473
CGAACCTAAC CGTAAAAACG

20

<210> 1474
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1474
CCAAATTAAA CCGAACTTTA CG

22

<210> 1475
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1475
TTGGGTAAAG TCGCGTAGC

19

<210> 1476
<211> 48
<212> DNA
<213> Artificial Sequence

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<400> 1476
GTCGCGAAGT TGTTTAAATT TCGTATTTGT AAGGTTTTGA TTTATCGT

48

<210> 1477
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1477
GTTCGTAAGG TTTTTCGGGA G

21

<210> 1478
<211> 24
<212> DNA
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<400> 1478
TTCGGGCGAT TCGGTCGTTC GTGT

24

<210> 1479
<211> 20
<212> DNA
<213> Artificial Sequence

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<400> 1479
CGAACCAACT CTAATCGCTC

20

<210> 1480
<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
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<400> 1480
GAACCAACTC TAATCGCTCG

20

<210> 1481
<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1481
TCGAACCAAC TCTAATCGCT

20

<210> 1482
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1482
TCGAACCAAC TCTAATCGCT C

21

<210> 1483
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1483
ACGATCAATA ACAACCGAAC AC

22

<210> 1484
<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1484
GCGTTTCGTT CGTAAGGTTT

20

<210> 1485
<211> 20
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<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1485
GCGTATTTTCG GAGAAGAAGT

20

<210> 1486
<211> 23
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1486

TAGTCGTTTA GGAGAGTTTT TCG

23

<210> 1487
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1487
GATTCGGTCG TTCGTGTTC

19

<210> 1488
<211> 24
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1488
AACACCGCGA CTATTATACG ACTA

24

<210> 1489
<211> 23
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1489
TTCGGTTGTT ATTGATCGTT TTA

23

<210> 1490
<211> 19
<212> DNA
<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1490
CTTCCGAACC CCGACTAAT

19

<210> 1491
<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1491
AATTATTAGT CGGGGTTCGG

20

<210> 1492
<211> 31
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1492
AGCGCGTTCG GAAATCGAGG TTTTATTAAG T

31

<210> 1493
<211> 23
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1493
TACTCGACTC CGAAAACAAC TAA

23

<210> 1494
<211> 20
<212> DNA
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<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1494
CCTCGAACCA ACTCTAATCG

20

<210> 1495
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1495
CGATCAATAA CAACCGAACA C

21

<210> 1496
<211> 23
<212> DNA
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<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1496
 GTTAGTTAGG TTCGTAGGCG TTA

23

<210> 1497
 <211> 19
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<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1497
 TCGTAAGGTT TTTCGGGAG

19

<210> 1498
 <211> 21
 <212> DNA
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<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1498
 GGTAGTGCGA GCGATTAGAG T

21

<210> 1499
 <211> 20
 <212> DNA
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<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1499
 TTCGTAAGGT TTTCGGGAG

20

<210> 1500
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220> bisulfite treated
 <223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
 <400> 1500
 CGAGGTTTTT GTAGGTCGC

19

<210> 1501
 <211> 22

<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1501
TTTTTGTTGT TCGCGTTAAG TC

22

<210> 1502
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1502
GTTTCGGGTT GTAAAGTTTC G

21

<210> 1503
<211> 24
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1503
TTGGGTTAAG TCGCGTAGCG CGGT

24

<210> 1504
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1504
AGTAATTTTG TTTCGGGTCG T

21

<210> 1505
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1505
GAACCTAACC GTAAAAACGC C

21

<210> 1506
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1506
CTACAAAAC CTCGACCCG

19

<210> 1507
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1507
GTTTCGTGTTT GGTGTTATT GA

22

<210> 1508
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1508
ATTTTATTC CCCGCTAACG A

21

<210> 1509
<211> 23
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1509
ATTTCGGTAA ATTCGTAGGT TTT

23

<210> 1510
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1510
ACCAACTACG TCGAACCAA

19

<210> 1511
<211> 24
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1511
GTTGGGTCGT AAGTTTATA GTCG

24

<210> 1512
<211> 23
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1512
TAATTTTGT TGTTCGCGTT AAG

23

<210> 1513
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1513
CTCGACTCCG AAAACAATA AC

22

<210> 1514
<211> 18
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1514
TCGGGAGTTA CGGTTTGA

18

<210> 1515
<211> 22
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1515
GATAACGCCT ACGAACCTAA CT

22

<210> 1516
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1516
CGGGTCGAGG TTTTGTAG

19

<210> 1517
<211> 37
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1517
GGTTTGTATT TATCGTTGTT TTTTCGTAAA GTTCGGT

37

<210> 1518
<211> 24
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1518
TTAGTCGTTT AGGAGAGTTT TTCG

24

<210> 1519
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1519
CGAAACCTAC TCGACTCCG

19

<210> 1520
<211> 31
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1520
GATCGTTTTA ACGTCGTTAG GGACGATTCG T

31

<210> 1521
<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1521
TCGTGTTCGG TTGTTATTGA

20

<210> 1522
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1522
CCAACTACGT CGAACCAAA

19

<210> 1523
<211> 20
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1523
TTTTTATTCC CCGCTAACGA

20

<210> 1524
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1524
ACCTCGAACC AACTCTAATC G

21

<210> 1525
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1525
CGGTTTTTAG GGTGAGTCG

19

<210> 1526
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1526
CGCGACCTAC AAAAACCTC

19

<210> 1527
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1527
GCGACCTACA AAAACCTCG

19

<210> 1528
<211> 21
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1528
GAAACCTACT CGACTCCGAA A

21

<210> 1529
<211> 19
<212> DNA
<213> Artificial Sequence

<220> bisulfite treated
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4
<400> 1529
CGTTCGTGTT CGGTTGTTA

19

<210> 1530
<211> 31
<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1530

ATCGTTTTAA CGTCGTTAGG GACGATTCGT T

31

<210> 1531

<211> 23

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1531

AAACGAACGA CCAATAAAAA CAT

23

<210> 1532

<211> 22

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1532

AACGATCAAT AACAACCGAA CA

22

<210> 1533

<211> 24

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1533

TACGACTATA AACTTACGA CCCA

24

<210> 1534

<211> 37

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1534

TTACGATGGC GGGGCGGCGG CGCGAATATA TAAAGGA

37

<210> 1535